M.D. Thesis.

Experimental Splenectomy In Certain Protozoan Infections, With Especial Reference To Feline Piroplasmosis.

By

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Seven Microphotographs.

I

Introduction

Since Paley in his "Natural Theology" enunciated the opinion that "The spleen may be merely a stuffing to fill a vacancy or hollow", a considerable volume of knowledge concerning this organ has accumulated. Our understanding of the distinctive functions of the spleen however, has not yet been placed on as firm a basis as that of many other organs of the animal body.

Gray (1854) opined that "the function of the spleen is to regulate the quantity and quality of the blood". This contention has recently received strong support from the experiments of Barcroft and his colleagues (Barcroft, 1925; Barcroft, Harris, Orahovats and Weiss, 1925). The formation of the lymphocytes would appear to be a function of the spleen(Jolly, 1923). The spleen has long been regarded as the grave of worn-out red blood-corpuscles. The experiments of Eppinger and Ranzi (1920), Bolt and Heeres (1922) and of Mann, Theard, Bollman and Baldes (1928) afford much evidence in support of this view. Other functions that have been attributed to the spleen are the secretion of a hormone regulating the activity of the haemato-poietic tissue of the marrow (Leake and Bacon

1924); and the formation of enzymes which play a part in the metabolism of the purine bodies (Jones and Austrian 1906).

The function of the spleen with which this thesis is concerned however, is its participation in the defence of the body against micro-organisms of disease. Views have been advanced that the mechanism of this activity may be both cellular and humoral.

There exists a certain amount of experimental evidence to indicate that the spleen may play a part in the production of humoral antibodies. Pfeiffer and Marx (1898) found that bacteriolysins were more concentrated in the spleens than in the sera of immunized guinea-pigs.

Deutsch (1899) found that splenectomy of guinea-pigs prior to inoculation of typhoid vaccine did not materially affect antibody production, but that the operation when performed during the immunizing period caused a definite decrease in agglutination titres as compared with controls. Luckhardt and Becht (1911) showed that splenectomy of dogs lessened the titres of haemolysins and haemagglutinins against foreign red blood-corpuscles. Tsurumi and Kohda(1913) found that in rabbits immunized against B. typhosis, complement fixing

antibodies appeared first in spleen extracts. Bieling and Isaac (1921) demonstrated that "blocade" of the reticulo-endothelial system exercised an inhibitory influence on haemolysin formation. Taliaferro (1924), and Regendanz and Kikuth (1927) have shown that an antibody against Trypanosoma lewisi normally present in the white rat is deficient in animals from which the spleens have been removed.

It seems probable however, that it is with immunity on a cellular basis that the defence function of the spleen is mainly concerned. It has long been known that cells of the spleen may be actively phagocytic to foreign bodies, both inanimate and animate, that may pass through the organ. Hoffman and von Recklinghausen (1867) showed that particles of carmine injected into animals intravenously were deposited in the spleen. Nearly thirty years later Muscatello (1895) demonstrated a similar phenomenon with carmine injected into the peritoneum. Buxton and Torrey (1906) confirmed this in the case of carbon particles. Eppinger and Ranzi (1920) state that in pyrodin poisoning many of the red blood-corpuscles contain small granules- Heinz bodies - and that these corpuscles are effectively filtered off by the spleen. Numerous experiments have satisfactorily demonstrated that bacteria can be filtered off from

the circulation by the spleen. Bordet (1895) showed that in an infected animal bacteria were present in greater numbers in the spleen and liver than in the heart blood. Bail (1905), and Buxton (1906) demonstrated that typhoid bacilli injected into rabbits became distributed mainly in the liver and spleen. Kyes (1916) found that pneumococci injected intra-venously into the naturally resistant pigeon became localized in the macrophage cells of the spleen and liver, and Nagao (1920) obtained similar results with dead staphylococci injected into rabbits. Similar results have been obtained in perfusion experiments with isolated spleens. Ozaki (1917) perfused an isolated dog's spleen with a suspension of staphylococci and observed extensive phagocytosis. Manwaring and Frischen (1923) obtained similar results with spleen and liver. That retention of bacteria by the spleen does not necessarily involve their death has been proved by Topley and Wilson (1923) who recovered Bacillus aertrycke from the spleens of mice several weeks after recovery from non-fatal infections.

The study of the effect of splenectomy on the resistance of animals to infection has been pursued now for some thirty years. The entire removal of the spleen would, of course, withdraw both its cellular and - if any - its humoral contribution to the immunity of the body.

The modern conception of a unified system of phagocytic cells, the reticulo-endothelial system of Aschoff (1924), recognizes that the distribution of the sessile macrophages within the body varies in different animal species. The size of the spleen relative to that of the body as a whole differs considerably in different species, so the actual importance of the spleen in the phagocytic defence of the body may be expected to vary accordingly. For this reason, experiments based on the effect of splenectomy on the resistance of an animal to infection might reasonably be expected to be more decisive in the case of animals having a high spleen-body weight ratio. According to Cannon and McClelland (1929) this ratio is highest in dogs and rats.

These splenectomy experiments have been more numerous and the results more decisive in the case of infections due to certain protozoa than in those due to bacteria. Bardach (1889) inoculated anthrax bacilli into the blood stream of dogs from which the spleens had previously been removed. The dog is naturally resistant to anthrax, but these desplenated dogs succumbed. Malm (1890) confirmed these results, but Melnikow-Raswedenkow (1896) failed to do so. Werigo (1894) studying anthrax septicaemia considered that the spleen

played a minor rôle in the body's defence. Zinsser (1923) found that splenectomized guinea-pigs were not more susceptible to tubercle bacilli than were normal controls.

In the case of spirochaete infections Soudakewitch (1891) found that Spirillum obermeieri caused a non-fatal infection in normal monkeys from which they eventually completely recovered; in the case of monkeys from which the spleens had previously been removed the spirochaetes multiplied abundantly with fatal results. More recently Regendanz (1928) removed the spleens from Brazilian opossums which then developed a fatal spirochaete infection which had presumably been latent. Meleney (1928) observed similar relapses in spirochaete infections following splenectomy of the grey squirrel and of the striped chipmunk.

During the last five years numerous experiments have been described on the significance of splenectomy in connection with Bartonella infections. Lauda (1925) found that white rats following splenectomy developed a severe and frequently fatal anaemia. Mayer, Borchardt and Kikuth (1927) established that this anaemia was caused by the small rod-like intra-corpuscular organisms now known as Bartonella muris-ratti. That the effect of splenectomy is to activate a previously existing

latent infection has since been established by the experiments of numerous workers. (Kikuth 1928, Bayon 1928, Ford and Elliot 1928, Schilling 1928). Kikuth (1928a), described a Bartonella canis which appears in the dog following splenectomy and causes a severe anaemia.

Turning now to the effect of the splenectomy on the course of infection due to protozoa proper, with which this thesis is particularly concerned, it is found that the relevant literature is considerable. It is convenient to consider first that relating to trypanosome infections. Bradford and Plimmer (1902) studied the course of infection of Trypanosoma brucei in rabbits, dogs, cats and rats from which the spleens had been previously removed, and they found that these desplenated animals died more quickly than did control animals. Laveran and Mesnil (1902) however claimed that removal of the spleen from a white rat did not influence a subsequent T. brucei infection. Sauerbeck's (1905) experiments supported Bradford and Plimmers' claims, and so did those of Rodet and Vallet (1906). But Laveran and Thiroux (1907) working with T.evansi in rats and guinea-pigs were unable to detect any difference in the course of infection of splenectomized animals; they concluded that the spleen

merely removed disintegrated bodies of the parasites after the trypanolytic crisis. Rodet and Vallet (1907) continuing their work maintained that in the case of T. brucei there is actual destruction of the parasites in the spleens of rats and guinea-pigs. Massaglia (1907), and Gottberg (1908) both reported negative results, and were unable to attribute to the spleen any special protective function in trypanosome infections. During the next twenty years but little work on the subject was published. Krumbhaar (1928) reported that splenectomy of dogs accelerated the course of a subsequent T. equiperdum infection. Kligler (1929) studied the effect of splenectomy on white rats infected with T. evansi. He found that the splenectomy activated intense Bartonella anaemia and therefore treated the rats with Salvarsan which protected them from the Bartonella. He reported a complete absence of resistance to infection in splenectomized rats when the virus was injected into the peritoneum, but when the inoculum was administered subcutaneously the effect of splenectomy was insignificant. Linton (1929) reported that splenectomy had no effect on the duration of life of guinea-pigs infected with T. equiperdum. Reference has already been made to the work of Regendanz and Kikuth (1927) who demonstrated that the reaction product present in normal T. lewisi infections is formed principally in

the spleen.

It will be seen that in the case of experimental trypanosomiasis the results of splenectomy are anything but clear-cut. This is perhaps more readily understood when it is realised how really small has been the experimental material relative to the number of variables at stake. Trypanosomes differing in species and in strain have been employed in the production of artificial diseases in various laboratory animals. In some cases, conditions approximating to natural disease are obtained: there is some interplay between the virulence of the particular strain of trypanosome and the resistance of the particular host. In others of these laboratory infections, however, the tissues of the host merely behave as a nutrient medium in which the parasites multiply without let or hinderance. This is the case with many strains of T. brucei in rats. (Laveran and Mesnil 1907). It is noteworthy that the most clear-cut results of splenectomy have been in rats infected with T. lewisi, the only natural form of trypanosomiasis experimented upon in this connection.

In the case of Sporozoa infections, the results of splenectomy have been much more decisive. Gonder and Rodenwalt (1910) found that Plasmodium kochi, a natural parasite of the ape,

caused much heavier infections in splenectomized apes than in normal controls, and that in cases of latent infection, relapse would follow splenectomy. The same authors also reported that dogs recovered from canine piroplasmosis relapsed after splenectomy. Ciuca (1912) also working with Babesia canis (the parasite of canine piroplasmosis) stated that when the dogs had completely recovered from the infection removal of the spleen did not cause a relapse, but that when performed during the course of the disease the operation aggravated the condition. De Kock and Quinlan (1926) splenectomized South African sheep, these subsequently developed a light infection of Babesia ovis. The same authors (De Kock and Quinlan 1926a) reported that splenectomy performed on horses which were carriers of Babesia equi, and on cattle which were carriers of Babesia bigemina and of Theileria mutans, in all cases caused severe relapses. The operation produced no such effect on uninfected animals, but when these animals were subsequently infected with the appropriate disease it ran an unusually severe course. Kikuth (1927) showed that splenectomy of African monkeys (Ceropithecus) activated latent infections of Babesia rossi. Meleney (1927) noted the appearance of species of Babesia after removing the spleens from squirrels. Nauck (1927) removed the spleens from the grey

squirrel Sciurus vulgaris and reported the appearance of Babesia and Haemogregarines not in evidence before the operation. Noguchi (1928) found splenectomy of macacus monkeys caused malarial relapses, thought to be due to Plasmodium inui. Regendanz and Kikuth (1928) observed the activation of latent infections of Babesia braziliensis in splenectomized opossums. The writer, recorded in a preliminary notice (Davis 1929,) that Babesia felis a natural parasite of the wild cat produced benign infections in normal domestic cats but very severe ones in cats which had been desplenated. Adler (1930) states that a non-pathogenic Babesia of the rodent Meriones tristrami multiplies and becomes pathogenic after splenectomy.

In the unanimity of these results, they afford a striking contrast to those obtained in experimental trypanosomiasis. It is significant that in all these cases of sporozoal infection the host-parasite relationship has been that obtaining under conditions of natural infection.

A function of the spleen that can only be mentioned here, is the part it may play in chemotherapy. The experiments of Kritschewsky and Meersohn (1920), of Kopilow (1927), of Feldt and Schott (1927) and of Kritschewsky (1927) indicate that the therapeutic efficiency of the appropriate

drug in streptococcal, spirochaetal and in trypanosome infections is definitely impaired by splenectomy, with or without "blocade" of the reticulo-endothelial system.

It is not proposed to refer in detail to the various papers dealing with the effect on resistance of blocade of the reticulo-endothelial system by means of the intra-venous injection of colloidal suspensions. The results are conflicting, largely it would seem on account of the lack of uniformity in the reaction of different animals to this blocade. In some cases a moderate dosage of colloid would seem to "block" the reticulo-endothelial system effectively, in other cases a similar dosage merely stimulates the cells in question into greater activity (Standenth 1923). Most of these experiments have had for their object the investigation of the effect of blocade on the ability of the animal to form specific antibodies; relatively few have been concerned with its effect on resistance in experimental infections. Wright (1927) found that the ability of rabbits to dispose of pneumococci injected intra- venously was not affected by preliminary injections of a suspension of India ink. Jungeblut (1928) found no evidence that blocade of mice lowered their resistance to

pneumococci. Cannon and McClelland (1928) however, did find that very large doses of India ink led to the production of Bartonella anaemia in rats.

II

Experimental Feline Piroplasmosis

In 1928 the writer discovered a small piroplasm in a wild cat (Felis ocreata Gmelin). The name Babesia felis was proposed for the parasite. In the published description of it (Davis, 1929) a preliminary account was given of its ability to produce benign infections in normal domestic cats and severe infections in splenectomized ones.

It is now proposed to record in greater detail the observations that have been made during the last two years on these experimental infections with special reference to the effect of splenectomy. Over sixty cats have been infected with the parasite. In some of these the infection has been closely and continuously studied over periods of some months, in other circumstances have rendered this impossible, but on the whole sufficient data have been collected to justify the drawing of certain definite conclusions. It is deemed desirable to record the course of the infection in normal cats in some detail in order that the departure from this course initiated by splenectomy may be more readily appreciated.

Since the inception of this work the objective has changed somewhat. Earlier, the interest centred mainly on the parasite itself and

investigations were directed to elucidating its life-cycle, searching for schizogony forms et cetera. Latterly however, the work has been concerned more with the reaction of the host, i.e. the pathology and immunology of the infection. It may be stated here that sexual forms of the parasite have never been encountered in the cat. The parasite so far as is known multiplies in its tissues by simple division, presumably into four by means of the cross arrangement.

The methods employed in studying feline piroplasmosis were simple. The effect of the infection on the host was assessed by recording obvious departures from health such as may be indicated by loss of condition, abnormalities of the urine, faeces and temperature, and by making frequent haematological observations. In the event of death post-mortem examinations were of course made whenever practicable. The progress of the infection itself was measured by counting the relative number of red blood-cells parasitized. This was done as follows. Blood films were made by spreading a drop of blood from the ear vein of the cat on a perfectly clean slide by means of a spreader made from a square cover-slip. The blood was drawn along, not pushed. In this manner the thickness of the resulting film could be regulated by controlling the angle between the spreader and

the slide. Latterly Leishman's stain has been exclusively used as it is simple to use and uniform in its results. A diaphragm with a square aperture was fitted to the X 10 ocular of the microscope, using it, it was found that with the 1/12 inch oil immersion lens and the draw-tube at 155 mm, the field would comprise two hundred red cells in the case of films or portions thereof which satisfied certain arbitrary criteria. Only films were used for this purpose in which the corpuscles were spread perfectly flat and compactly, but not actually overlying each other. In the case of light infections twenty-five or fifty such fields would be examined and the number of infected corpuscles counted. In this way approximately five or ten thousand corpuscles could be rapidly surveyed. It is realized that this method is not strictly accurate, but it is considered adequate in dealing with very light infections where it is desirable to inspect large numbers of corpuscles. The number of infected corpuscles thus determined is expressed as a percentage of the total number examined. Trial estimations indicated that the error rarely exceeded ± 0.02%. In the case of heavy infections a smaller number of corpuscles were examined and these were actually counted. For this purpose thinner films were employed in which the corpuscles could be

more readily counted. On each occasion two to five hundred corpuscles would be counted and the percentage of infected ones determined.

The cats themselves were local bazaar cats caught in traps. Only tame ones were used. They were kept in separate cages and fed on raw meat daily. The animals kept in excellent condition on the whole, but there were several epizootics, presumably of feline distemper, which were accompanied by a high mortality rate, and deaths would, from time to time, occur which appeared to be due to enteritis.

The inoculations were usually performed by mixing a few drops of blood from an infected cat with an equal amount of 2% sodium citrate solution. The mixture was then inoculated subcutaneously, intra-peritoneally or intra-venously.

The Course of Infection in the Normal Cat.

Protocols are appended of nine cats on which observations have been made. After inoculation of small quantities of infected blood the incubation period elapsing before the appearance of the parasite in the peripheral blood varied from twelve to twenty-five days. It will be seen that during the month immediately following the appearance of the parasites the percentage of infected corpuscles steadily rises until within the

neighbourhood of one per cent are infected. Thereafter the percentage falls. For the rest of the cat's life a small number of red cells - usually between 0.1 and 0.5 per cent always appear infected. In the protocols the second place of decimals is used only in the case of very light infections. On account of the method of the estimation being of only approximate accuracy, the use of the second place of decimal in moderate infections was not considered to serve any useful purpose. When no infection is noted it is implied that no parasites were seen in ten thousand red cells examined.

No evidence was obtained to suggest that these light infections ever caused any ill effect in the cats. Haematological observations indicated that the red cell-count was quite uninfluenced by the infection. The white cell-counts however did show a tendency to rise during the course of the infection. It will be seen however that the rise is not particularly significant. The differential white cell-counts fail to reveal any significant departure from the normal. At this point it is convenient to consider the normal haematological findings of the cat. The findings of various authors may be tabulated as follows :-

Author	Average count for cats	
	Red Cell	White Cell
Hayem (1889)	9,900,000	7,200

Author	Average count for cats	
	Red Cell	White cell
Sherrington (1894)	6,800,000	14,017
Busch and Van Bergen (1902)	6,600,000	13,331
	Polymorphonuclears	55.5%
	Lymphocytes	34.38%
	Mononuclears	4.89%
	Eosinophils	5.2%
	Mast cells	.035%
Goodall (1910)	8,000,000	18,000
	Polymorphonuclears	54%
	Mononuclears	37%
	Eosinophils	9%
Burnett (1917)	6,500,000	13,300
Kleineberger (1927)	7,393,000	15,600
	Polymorphonuclears	68.5%
	Lymphocytes	25%
	Mononuclears	0.04%
	Eosinophils	5%
	Mast cells	1.46%
Sanders (1928)	5,732,666	10,246

It will be seen that the findings recorded in the protocols are of the same order as most of these already recorded. The average initial red count of fifteen Khartoum cats was 7,244,000 per cn. mm. and the average white count of eleven cats was 16,477.

In the earlier part of these studies interest was taken in the small spherical chromatin granules which were observed in a small percentage of red cells in all the local cats examined. The bodies closely resemble the Anaplasma marginale of Theiler (1910). Jowett (1911) recorded similar

marginal points in cats. During the course of the observations on the piroplasm the percentage of corpuscles containing these Howell-Jolly bodies was determined on many occasions. The results show a remarkable constancy, for, irrespective of the degree of piroplasm infection, the percentage of corpuscles containing these Howell-Jolly bodies seldom varied more than from 0.1 to 0.2 per cent. It was concluded that these bodies were not parasites and bore no relation to the babesia.

Cat No. 2.

Female, black 1200 gms.

Date	% of Red Cells infected	
3.3.28		Inoculated intravenously with 0.2 cc citrated blood from No.1 (original wild cat)
5.3.28	0	
7.3.28	0	Blood Count R = 6,750,000
9.3.28	0	
11.3.28	0	Temp. 101.8 °F.
13.3.28	0	
15.3.28	0	
17.3.28	0.03	Temp. 101.7°F.
19.3.28	0.07	
21.3.28	0.1	
23.3.28	0.1	Temp. 102.1 °F.
25.3.28	0.3	Blood Count R = 6,900,000
27.3.28	0.7	
31.3.28	1.1	
2.4.28	0.8	Temp. 101.6 °F.
7.4.28	0.1	
13.4.28	0.2	
17.4.28	0.1	Temp. 101.9 °F.
24.4.28	0.1	Blood Count R = 7,350,000
5.5.28	0.1	Temp. 102.1 °F.
14.5.28	0.05	
19.5.28	0.03	
16.6.28	0.07	Blood Count R = 7,500,000
27.6.28	0.06	

No further examination possible. The cat died two months later during the writer's absence.

Cat No. 3.

Male, black, 850 gms.

Date	% of Red Cells infected	
3.3.28	Inoculated subcutaneously with 0.2 cc citrated blood from No. 1.	
5.3.28	0	
7.3.28	0	Blood Count R = 5,850,000
9.3.28	0	
11.3.28	0	Temp. 102.3 °F.
13.3.28	0	
15.3.28	0	
17.3.28	0	Temp. 102.1 °F.
19.3.28	0.05	
21.3.28	0.1	
23.3.28	0.1	Temp. 102.0 °F.
25.3.28	0.2	Blood Count R = 6,350,000
27.3.28	0.3	
31.3.28	0.6	
2.4.28	0.7	Temp. 101.9 °F.
7.4.28	1.3	
13.6.28	0.3	
17.4.28	0.2	Temp. 102.1 °F.
20.4.28	Died - apparently from enteritis.	

Cat No. 5.

Male, white and sandy, 900 gms.

Date	% of Red Cells infected.	
17.4.28	Inoculated Intra-peritoneally with 0.5 cc of citrated blood from No. 6.	
24.4.28	0	Blood Count R = 7,400,000
30.4.28	0	
3.5.28	0	
5.5.28	0.02	
14.5.28	0.05	
19.5.28	0.8	
26.5.28	1.4	Blood Count R = 8,150,000
2.6.28	1.2	
10.6.28	0.8	
16.6.28	0.5	Blood Count R = 8,300,000
27.6.28	0.2	

No further examinations possible, the cat died two months later during the writer's absence.

Cat No. 8

Male, white and tabby, 750 gms.

Date	Inoculated Intra-peritoneally with 0.5 cc citrated blood from No.5.
	% of Red Cells infected.
7.6.28	0
10.6.28	0
12.6.28	0
14.6.28	0.02
16.6.28	0.07
19.6.28	0.1
21.6.28	0.1
25.6.28	0.3
27.6.28	0.5

Cat died two months later in writer's absence.

Cat No. 11

Male, white and tabby, 950 gms. Temp. 102.1 °F.

Date		
28.11.28	Blood Examination R = 8,100,000 Hb.=75% W = 21,200 Polymorphonuclears 75% Lymphocytes 22% Large Mononuclears 2% Eosinophils 1% H-J bodies present in 0.14% of corps. Inoculated subcutaneously with 0.5 cc. citrated blood from cat No. 6.	
	Percentage of Red Cells infected.	
6.12.28	0	
8.12.28	0	
10.12.28	0	
12.12.28	0	
13.12.28	0.01	
15.12.28	0.03	Temp. 101.7 °F
17.12.28	0.08	
19.12.28	0.1	
21.12.28	0.2	Temp. 102.2 °F.
23.12.28	0.2	Blood Examination R = 7,950,000 Hb = 75%
24.12.28	0.3	
26.12.28	0.4	Temp. 101.9 °F.
28.12.28	0.4	
30.12.28	0.6	Blood Examination R = 8,350,000 Hb = 75% W = 19,400 Polymorphonuclears 73% Lymphocytes 23% Large Mononuclears 3% Eosinophils 1% H-J. bodies in 0.2% of corpuscles
1.1.29	0.8	
3.1.29	0.9	
5.1.29	0.7	
7.1.29	0.6	
9.1.29	0.4	Temp. 101.8 °F.
11.1.29	0.6	
13.1.29	0.4	
15.1.29	0.3	Blood examination R = 8,200,000 Hb = 75%
19.1.29	0.3	

Cat No. 11 Continued

22.1.29	0.2	Temp. 102.4 °F.
26.1.29	0.1	
29.1.29	0.19	
1.2.29	0.21	Blood Examination
3.2.29	0.09	R = 8,450,000
		W = 23,200
5.2.29	0.1	Polymorphonuclears 65%
		Lymphocytes 30%
9.2.29	0.06	Large Mononuclears 3%
		Eosinophils 2%
		H-J. bodies in 0.13% corpuscles
12.2.29	0.05	
15.2.29	0.07	
19.2.29	0.1	Temp. 101.5 °F.
24.2.29	0.1	
28.2.29	0.1	Blood Examination
		R = 7,500,000
7.3.29	0.2	W = 19,400
		Polymorphonuclears 72%
14.3.29	0.3	Lymphocytes 24%
		Large Mononuclears 3%
21.3;29	0.2	Eosinophils 1%
		H-J. bodies in 0.17% corpuscles
30.3.29	0.2	
11.4.29	0.3	Temp. 102.2 °F.
18.4.29	0.4	
25.4.29	0.3	
2.5.29	0.2	Blood Examination
		R = 8,100,000
16.5.29	0.5	W = 22,000
		Polymorphonuclears 70%
23.5.29	0.3	Lymphocytes 26%
		Large Mononuclears 3.5%
30.5.29	0.4	Eosinophils 0.5%
		H-J. bodies in 0.2% corpuscles
6.6.29	0.5	
20.6.29	0.3	Temp. 101.7 °F.
27.6.29	0.3	
4.7.29	0.4	Blood Examination
		R = 7,350,000
11.7.29	0.2	W = 24,200
		Polymorphonuclears 76%
		Lymphocytes 22%
		Large Mononuclears 1%
		Eosinophils 1%
		H-J. bodies in 0.2% corpuscles

Cat died in September in writer's absence.

Cat No. 13.

Male, black and white 2050 gms. Temp. 101.5 °F.

Date		
28.11.28	Blood Count R = 7,600,000 Hb = 70% W = 16,200 Polymorphonuclears 68% Lymphocytes 27% Large Mononuclears 3% Eosinophils 2% H-J.bodies present in 0.22% of corps. Inoculated intravenously with 0.5 cc of citrated blood from No. 6.	
	% of Red Cells infected	
6.12.28	0.	
8.12.28	0	
10.12.28	0	
12.12.28	0	
13.12.28	0	
15.12.28	0.03	Temp. 101.3 °F.
17.12.28	0.05	
19.12.28	0.1	
21.12.28	0.08	Temp. 102.1 °F.
23.12.28	0.1	Blood Count R = 7,800,000 Hb. = 70%
24.12.28	0.1	
26.12.28	0.2	Temp. 102.3 °F.
28.12.29	0.3	
30.12.29	0.5	Blood Count R = 7,250,000 Hb. = 70% W = 18,400 Polymorphonuclears 72% Lymphocytes 23% Large Mononuclears 4% Eosinophils 1% H-J.bodies present in 0.18% of crops.
1.1.29	0.7	
3.1.29	0.7	
5.1.29	0.8	
7.1.29	0.9	
9.1.29	0.7	Temp. 101.7 °F.
11.1.29	0.5	
13.1.29	0.4	
15.1.29	0.4	Blood Count R = 6,700,000 Hb. = 70%
19.1.29	0.5	

No. 13 continued.

Date	% of Red corps. infected	
22.1.29	0.3	
26.1.29	0.1	
29.1.29	0.1	
1.2.29	0.08	Blood count
		R = 7,350,000 Hb. = 70%
3.2.29	0.05	W = 15,600
		Polymorphonuclears 70%
5.2.29	0.04	Lymphocytes 26%
		Large Mononuclears 2%
9.2.29	0.06	H-J. bodies present in 0.2% of corps.
12.2.29	0.08	
15.2.29	0.1	
19.2.29	0.2	Temp. 102.2 °F.
24.2.25	0.2	
28.2.29	0.1	Blood count
		R = 7,800,000 Hb. = 70%
7.3.29	0.17	W = 19,000
		Polymorphonuclears 76%
14.3.29	0.28	Lymphocytes 20%
		Large Mononulears 3%
21.3.29	0.1	Eosinophils 1%
		H-J.bodies present in 0.18% of corps.
30.3.29	0.1	
11.4.29	0.3	Temp. 101.5 °F.
18.4.29	0.1	
25.4.29	0.05	
2.5.29	0.1	Blood count
		R = 7,450,000
16.5.29	0.1	W = 17,600
		Polymorphonuclears 74%
23.5.29	0.1	Lymphocytes 21%
		Large Mononuclears 2%
27.5.29	Dead	Eosinophils 3%
		H-J. bodies present in 0.2% of corps.

Cat No. 11 died along with three others presumably poisoned.

Cat No. 20.

Female, white and sandy 1500 gms.

Date		
9.4.29	Inoculated Subcutaneously with 0.2 cc citrated blood from No. 12.	
	% of Red Cells infected.	
17.4.29	0	Blood Count R = 7,750,000
19.4.29	0	
21.4.29	0.03	
23.4.29	0.05	
30.4.29	0.5	
11.5.29	0.1	
13.5.29	0.3	
16.5.29	0.7	
21.5.29	0.9	Blood Count R = 8,100,000
26.5.29	0.3	
28.5.29	0.2	
30.5.29	0.3	
9.6.29	0.1	
17.6.29	0.5	
25.6.29	0.2	Blood Count R = 7,900,000
3.7.29	0.1	
11.7.29	0.05	

No further examinations possible. Cat died in October during writer's absence.

Cat No. 26.

Male, black, 900 gms.

Date	% of Red Cells infected.	
23.5.29	Inoculated Subcutaneously with 1.5 cc citrated blood from No. 6.	
30.5.29	0	
5.5.29	0	
9.6.29	0	Blood Count R = 6,650,000 W = 12,600
17.6.29	0.8	
25.6.29	2.3	Blood Count R = 6,900,000
3.7.29	.9	
8.7.29	.8	Blood Count R = 6,450,000 W = 13,400
11.7.29	.6	
4.11.29	.5	Blood Count R = 7,250,000 W = 16,200
8.12.29	.3	
17.12.29	.2	
29.12.29	.4	
10.1.30	Died along with several others during an exceptionally cold night.	

Cat No. 32.

Female, tabby, 1350 gms.

Date	% of Red Cells infected.	
11.7.29		Inoculated subcutaneously with 0.2 cc citrated blood from No. 24.
4.11.29	0.3	
8.12.29	0.3	Blood Count
		R = 7,500,000
14.1.30	0.07	W = 13,600
		Polymorphonuclears 74%
		Lymphocytes 21%
		Mononuclears 2%
5.4.30	0.03	Eosinophils 3%
14.4.30	0.02	Blood Count
		R = 6,950,000
		W = 14,800
		Polymorphonuclears 66%
		Lymphocytes 24%
		Mononuclears 3%
		Eosinophils 7%
26.7.30	0.1	
30.7.30	0.2	
3.8.30	0.3	Blood Count
		R = 7,150,000
5.8.30	0.4	W = 15,200
		Polymorphonuclears 74%
10.8.30	0.4	Lymphocytes 22%
		Mononuclears 2%
13.8.30	0.3	Eosinophils 2%
21.8.30	0.1	
31.8.30	0.1	Blood Count
		R = 6,750,000
		W = 17,600
		Polymorphonuclears 73%
		Lymphocytes 22%
		Mononuclears 3%
		Eosinophils 2%

The Course of the Infection in Spleenless Cats.

The technique of splenectomy of the cat was found to be quite simple. Anaesthesia was induced with chloroform and subsequently maintained with ether. The abdomen of the animal having been shaved an incision one inch in length over the left lumbar quadrant readily allowed the withdrawal of the spleen. The pedicle would then be clamped and ligated in two stages. Two rows of cat-gut sutures were employed in suturing the wound, which was then finally painted over with ten per cent iodine in collodion. The mortality rate was very small and secondary sepsis occurred in only one case.

According to Cannon and McClelland (1929) the spleen of the cat weighs only 0.05% of the total body-weight. In fifteen healthy Khartoum cats that were consecutively operated upon however, the average ratio of spleen-weight to body-weight worked out at 0.189%, the maximum being 0.28% and the minimum 0.08%. Whether this greater size of spleen in the Khartoum cat is a result of environment it is difficult to say. Unfortunately this systematic weighing was not adopted earlier, and the spleens of only two of the cats infected with Babesia felis were weighed. In No. 39, it weighed 0.4% of the total body weight. It would be interesting to see if splenic hypertrophy is the

rule in infected cats.

Before going on to consider the effects of splenectomy on infection, it is as well to review briefly the recognised sequelae in healthy animals. Pierce (1917) states that in dogs the operation causes a fall in the red blood-count which however returns to normal in three months. Freytag (quoted by Eppinger 1920) found that there was an immediate red cell increase followed by a fall and then a gradual return to normal. Nägeli (1923) states that a polycythaemia may ensue. This author also holds that normoblasts may be a permanent feature of the blood picture after splenectomy. Pierce (1917) however states that their appearance is inconstant in splenectomized dogs. Hirschfeld (1915) mentions Howell-Jolly bodies as a permanent aftermath in splenectomy, again Pierce fails to confirm. Kurloff (quoted by Eppinger 1920) found that splenectomy of guinea-pigs caused a permanent leucocytosis, the increase affecting the lymphocytes chiefly. Musser and Krumbhaar (1913) also record a leucocytosis following the operation in dogs, and De Kock and Quinlan (1926a) did so in sheep. Pierce (1917) states that in dogs splenectomy is followed by a rise in the white cell-count which then falls to normal. Naguchi (1912) found in man a post-operative lymphocytosis and a late eosinophilia.

Other effects of splenectomy are hypertrophy of the lymphatic glands (Nägeli 1923), thrombocytosis (Pierce 1917), delay in recovery from anaemia (Pierce1917), decreased tendency to jaundice (Pierce 1917, Eppinger 1920), and increased cholesterin content of the blood (Eppinger 1920).

In the present series of splenectomies, two cats were never infected in order that the effects of the operation itself might be observed. Reference to the protocols of Nos. 14 and 44 indicates that the chief result is an increase in the number of white blood-cells, not however materially affecting any particular variety.

The protocols of eleven de-splenated infected cats are appended. In six of these the splenectomies were performed after infection had been established. The remaining five cats were infected after the spleens had been removed.

The six cats (Nos. 6,7,18,23,31,39) that were already infected when operated upon reacted in many respects in an identical fashion. It will be seen that following the operation the degree of intensity of infection rose steadily during the ensuing three weeks. The percentage of infected corpuscles far exceeded that seen in normal cats. In Nos. 6 and 7 as many as ninety per cent eventually became infected. It will also be seen that when this number reached the

neighbourhood of forty per cent the red cell-count began to fall and during the next few days would rapidly drop to the neighbourhood of a million. The later protocols are more detailed as daily observations were made. The urine of three of the cats was temporarily port-wine coloured and revealed oxyhaemoglobin spectroscopically at the height of the illness. In the case of Nos. 31 and 39 although daily urine examinations were made, no blood pigment was found. In all cases heavy albuminuria was a feature of the stage of intense infection. It has been observed that normal cats have traces of albumen in the urine, but in the cats in question the amount of albumen far exceeded that normally seen. Temperature observations were made, it was noted that the temperature was never consistently hypernormal&tended to drop during the crisis. The blood picture during the height of infection was that of a very severe anaemia. Aniscytosis, poikilocytosis and basophilia were constant features. Normoblasts and megaloblasts appeared in the late stage in all cases. The haemoglobin percentage was recorded by the Tallquist scale. It will be noted that it remained relatively high, so giving a colour index of more than unity during the extreme stage of anaemia.

Of the six cats one died at the height of infection, three were killed for tissue examination and two survived. In the case of the survivors the percentage of infected cells rapidly fell, and the red cell-count correspondingly rose. During the fortnight succeeding the crises the degree of infection fell to about 4% and in the case of No. 6, which was observed for over a year afterwards, it remained at this level.

Before further considering the pathology of the condition it is convenient to review the course of events in the cats that were infected subsequent to splenectomy. This procedure has the advantage that the results cannot be attributed to the general effect of the operation, for the animals were not inoculated until they had quite recovered from its effects. It will be seen that following inoculation there is an incubation period of a fortnight to a month, then after the first appearance of the parasites in the peripheral blood they under go rapid multiplication and bring about a state of affairs similar to that already described. Of the five cats, four died and one survived the intense phase of infection.

Reviewing the series as a whole, it will be seen that the white cell-count in some cases shows a definite increase. The differential

counts do not on the whole show sufficient correlation in their variation to permit conclusions to be drawn, but it is considered suggestive that in those cases in which leucocytosis was observed, the percentage of mononuclears was high.

During the phase of intense infection small red-staining granules were invariably present within the cytoplasm of some of the lymphocytes and large mononuclears. Many of these granules bore a striking resemblence to the chromatin portions of the parasites. They were only in evidence when the infection was heavy. It is considered that in all probability they represent babesia in the process of being phagocytosed.

An interesting observation is that in those cats that survived, the white cell-counts were definitely higher than in those that succumbed during the crises. The following table illustrates the point

Cat No.	White count during crisis	% Mononuclears	Fate
6	42,000	39%	Survived
23	58,200	59%	=
24	44,600	33%	=
12	20,000	22%	Died
21	16,400	33%	=
31	30,800	34%	=
47	22,200	22%	=

From this table cats that were

killed are of course omitted. The numbers are probably too small to be statistically significant, but the findings are suggestive that the survival of de-splenated cats may be dependant upon their ability to produce leucocytes, particularly mononuclears. It is interesting to compare the protocols of No. 21 with No. 24 and of No. 23 with No. 31 as these two pairs were living under identical conditions and the observations on each pair were made on the same occasions.

The remaining data that have been collected concerning the pathology of the condition may be summarized as follows. Jaundice was never observed. In some cats a trace of bilirubin was occasionally found in the urine. Van den Berg tests performed on the sera of cats killed during the intense phase were negative both direct and indirect. At autopsies the only constant features were; pale fatty liver and pale kidneys, in both organs the Prussian Blue reaction was very definite. The marrow in the femur was always red and jelly-like. Microscopically the main features were fatty degeneration, cloudy-swelling and pigmentation of the liver and kidney. The latter organ in some cases exhibited a collection of plasma and debris within the tubules and glomeruli. The marrow of the long bones showed an erythroblastic reaction.

Comparing these findings with those of similar conditions caused by pathogenic piroplasms

a distinct similarity is noted. In Canine piroplasmosis, a severe progressive anaemia is the chief feature. The red cell-count steadily decreases and then comes a haemoglobinuric crisis and the red-count falls to less than two millions. Normoblasts may be numerous and leucocytes are usually much increased (Nocard 1902; Nocard and Motas 1902). Texas Fever (Babesia bigemina) is characterized by a very low red cell-count and the most intense phase of anaemia may or may not be accompanied by haemoglobinuria. There is a leucocytosis mainly affecting the lymphocytes (Smith and Kilbourne 1893).

It would seem probable then, that the pathological condition observed in de-splenated cats infected with Babesia felis is akin to a natural disease, and that normal cats owe their immunity to the functioning of their spleens.

Effect of Spleen Diet.

Three of the spleenless cats-No.23, 39 and 50 were each given daily a quarter of a pound of raw sheep's spleen. It will be seen that Nos. 23 and 39 were chronically infected prior to the removal of the spleens. No. 50 however, was infected after the operation. In no case could the spleen diet be said to have any definite effect in inhibiting the multiplication of the parasites or lessening the severity of the accompanying anaemia.

It was not intended to draw any conclusions on the influence a spleen diet might have in preventing a fatal issue as the number of cats necessary for such an experiment would necessarily be very large, if results of statistical significance were to be hoped for.

Cat No. 14. (uninfected)

Female, black and white, 800 gms.

Date	% of Red Cells infected.	
28.11.28	0	Blood Count R = 6,500,000 W = 17,600 Polymorphonuclears 66% Lymphocytes 32% Large Mononuclears 1% Eosinophils 1% H-J. bodies in 0.18% of corps.
1.12.28	0	Splenectomy performed
6.12.28	0	
10.12.28	0	
15.12.28	0	
19.12.28	0	
22.12.28	0	
26.12.28	0	Blood Count R = 5,750,000 W = 24,000 Polymorphonuclears 58% Lymphocytes 35% Large Mononuclears 4% Eosinophils 3% H-J. bodies in 0.2% of corps.
1.1.29	0	
5.1.29	0	
8.1.29	0	
12.1.29	0	
15.1.29	0	
22.1.29	0	
26.1.29	0	Blood Count. Inoculated subcutaneously from No. 11 R = 6,500,000 W = 34,200 Polymorphonuclears 60% Lymphocytes 35% Large Mononuclears 3% Eosinophils 2% H-J. bodies in 0.2% of corps.
2.2.29	0	
5.2.29	0	
9.2.29	0	
10.2.29	0	Cat died during an epizootic.

Cat No. 44. (uninfected)

Female, black, 1220 gms.

Date	% of Red Cells infected	
26.7.30	0	Blood Count
		R = 6,550,000 Hb = 70%
		W = 12,800
		Polymorphonuclears 72%
		Lymphocytes 23%
		Large Mononuclears 2%
		Eosinophils 3%
27.7.30	0	Splenectomy performed. Weight of spleen = 2.8 gms. i.e. 0.22% of body weight.
1.8.30	0	Blood Count
		R = 7,250,000 Hb = 70%
		W = 15,600
		Polymorphonuclears 77%
		Lymphocytes 15%
		Large Mononuclears 2%
		Eosinophils 6%
5.8.30	0	Blood Count
		R = 7,800,000 Hb = 70%
		W = 18,000
		Polymorphonuclears 72%
		Lymphocytes 21%
		Large Mononuclears 4%
		Eosinophils 3%
13.8.30	0	Blood Count
		R = 7,650,000 Hb = 70%
		W = 28,200
		Polymorphonuclears 74%
		Lymphocytes 22%
		Large Mononuclears 2%
		Eosinophils 2%
23.8.30	0	Blood Count
		R = 7,700,000 Hb = 70%
		W = 26,000
		Polymorphonuclears 72%
		Lymphocytes 24%
		Large Mononuclears 3%
		Eosinophils 1%
31.8.30	0	Blood Count
		R = 7,250,000
		W = 22,200
7.9.30	0	Blood Count
		R = 7,300,000 Hb = 70%
		W = 21,800
		Polymorphonuclears 65%
		Lymphocytes 30%
		Large Mononuclears 3%
		Eosinophils 2%

Cat No. 6

Male, white, 1750 gms.

Date		
20.3.28	Blood Count	
	R =	5,600,000
	W =	17,800
	Polymorphonuclears	64%
	Lymphocytes	29%
	Large Mononuclears	3%
	Eosinophils	4%
	H-J. bodies present in 0.2% of corps.	
	Inoculated intravenously with 0.2 cc citrated blood from No. 1.	

% of Red Cells infected.

27.3.28	0	
31.3.28	0	
3.4.28	0	
5.4.28	0	
7.4.28	0	
9.4.28	0.05	
13.4.28	0.1	
17.4.28	0.1	
24.4.28	0.1	
10.5.28	0.1	
16.5.28	0.05	Blood Count Splenectomy performed
		R = 4,900,000
20.5.28	0.2	W = 20,200
		Polymorphonuclears 67%
22.5.28	2.8	Lymphocytes 26%
		Large Mononuclears 2%
24.5.28	5.2	Eosinophils 5%
		H-J. bodies present in 0.2% of corps.
26.5.28	15.2	
1.6.28	40.	
4.6.28	60.	
7.6.28	90.	Blood Count R = 2,300,000) Albumen and
9.6.28	88.	) Oxyhaemoglobin
11.6.28	70.	) in urine

No. 6 continued

% of Red Cells infected.

Date	%	
13.6.28	65.	Blood Count
		R = 1,600,000
15.6.28	50.	W = 42,000
		Polymorphonuclears 58%
20.6.28	40.	Lymphocytes 37%
		Large Mononuclears 2%
		Eosinophils 3%
18.11.28	5.2	Blood Count
		R = 4,200,000
3.12.28	5.7	Blood Count
		R = 4,350,000
8.12.28	4.8	W = 46,000
		Polymorphonuclears 55%
22.12.28	4.8	Lymphocytes 37%
		Large Mononuclears 3%
5.1.29	3.9	Eosinophils 5%
		H-J. bodies in 0.2% of corps.
16.1.29	3.6	
5.2.29	3.2	
9.2.29	3.7	Blood Count
		R = 3,850,000
7.3.29	3.2	W = 64,000
		Polymorphonuclears 47%
30.3.29	2.7	Lymphocytes 50%
		Large Mononuclears 2%
		Eosinophils 1%
18.4.29	3.5	Blood Count
		R = 4,150,000
2.5.29	4.2	W = 63,200
30.5.29	2.6	
25.6.29	2.4	Blood Count
		R = 4,300,000
11.7.29	2.2	W = 62,000
		Polymorphonuclears 59%
		Lymphocytes 34%
		Large Mononuclears 3%
		Eosinophils 4%
		H-J. bodies in 0.18% of corps.

Cat died in October 1929 during writer's absence.

Cat No. 7.

Male, black, 1900 gms.

Date

2.6.28 Inoculated intra-peritoneally with 0.5cc. citrated blood from No. 5

% of Red Cells infected.

10.6.28 0

14.6.28 0

16.6.28 0.2

21.6.28 0.2

27.6.28 0.4

Blood Count
R = 7,350,000 Hb = 70%
W = 23,2000
Polymorphonuclears 68%
Lymphocytes 18%
Large Mononuclears 3%
Eosinophils 11%
H-J.bodies present in 0.12% of corps.

19.11.28 0.2

28.11.28 0.3

Blood Count
R = 7,600,000 Hb = 70%
W = 18,600
Polymorphonuclears 75%
Lymphocytes 17%
Large Mononuclears 3%
Eosinophils 5%
H-J.bodies present in 0.18% of corps.

1.12.28 0.3 Splenectomy performed.

3.12.28 0.3

6.12.28 0.8

8.12.28 2.6

Blood Count
R = 6,900,000 Hb = 70%
H-J. bodies in 0.2% of corps.

10.12.28 15.3 Temp. = 101.4 °F.

15-12-28 17.4

16.12.28 19.7

19.12.28 60.

22.12.28 90.

Blood Count Temp. = 99.8 °F.
R = 2,850,000 Hb = 35%
W = 42,000
Polymorphonuclears 78%
Lymphocytes 17%
Large Mononuclears 1%
Eosinophils 4%
Numerous nucleated red cells.

No. 7 Continued.

% Red Cells infected.

Date			
24.12.28	82.	Blood Count	Temp. = 99.5 °F.
		R = 1,250,000	Hb. = 30%
		W = 46,000	
		Polymorphonuclears	64%
		Lymhpocytes	27%
		Large Mononuclears	2%
		Eosinophils	7%
		Numerous nucleated red cells.	
30.12.28	50.	Blood Count	Temp. = 100.7 °F.
		R = 1,550,000	Hb. = 40%
5.1.29	48.	W = 43,800	
6.1.29	45.	Blood Count	Temp. = 100.5 °F.
		R = 1,350,000	Hb. = 35%
		W = 45,200	
		Polymorphonuclears	65%
		Lymphocytes	25%
		Large Mononuclears	5%
		Eosinophils	5%

The cat was killed for autopsy.

Macroscopically, the chief features were the pale fatty liver, the congested kidneys and the red jelly-like marrow in the long bones. Sections and smears were made of all the organs and were searched for schizogony forms of the parasite with negative results. The chief pathological findingsswere fatty degeneration and cloudy -swelling of the liver cells and cells of the tubular ep epithelium in the kidneys. Intra-cellular pigment deposits were present in both organs, particularly the liver. The femur marrow contained numerous megaloblasts and normoblasts.

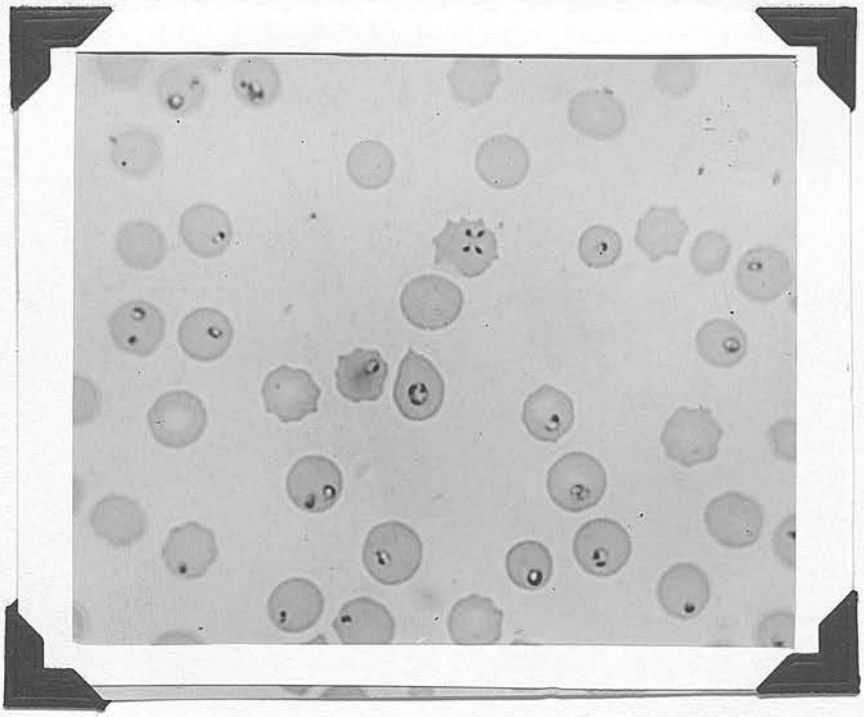


Figure 1.

Microphotograph of blood film of Cat No.7 on 19.12.28, showing heavy infection of Babesia felis. (× 1,400).

Cat No. 18.

Female, tabby, 900 gms.

Date	% of Red Cells infected.	
1.1.29	0	Blood Count R = 9,200,000 W = 10,800 Polymorphonuclears 58% Lymphocytes 28% Large Mononuclears 2% Eosinophils 12% H-J. bodies in 0.1% corps.
5.1.29	0	Inoculated Subcutaneously with 0.17 cc citrated blood from No. 13
8.1.29	0	
12.1.29	0	
15.1.29	0	
19.1.29	0	
22.1.29	0	
26.1.29	0.03	H-J. bodies in 0.1% of corps.
29.1.29	0.07	
30.1.29	0.1	Splenectomy performed.
2.2.29	0.3	
4.2.29	0.5	
5.2.29	1.7	
6.2.29	3.2	H-J. bodies in 0.1% of corps.
9.2.29	25.3	
10.2.29	45.0	Urine - albumen & oxyhaemoglobin
11.2.29	65.0	Blood Count Urine as above. R = 3,150,000 W = 27,200 Polymorphonuclears 77% Lymphocytes 20% Large Mononuclears 2% Eosinophils 1% Nucleated red cells fairly numerous.

Cat killed and examined for evidence of internal schizogony with negative results.

Cat No. 23.

Female, tortoiseshell, 1130 gms.

Date	% of Red Cells infected	
23.5.29		Inoculated subcutaneously with 1.5 cc. citrated blood from No. 6.
9.6.29	0.1	
17.6.29	1.2	
25.6.29	1.0	
3.7.29	0.6	
8.7.29	0.3	
12.7.29	0.2	
4.11.29	0.1	
14.11.29	0.2	
5.4.30	0.06	
14.4.30	0.02	
26.7.30	0.04	Henceforth Cat was given ¼ lb. raw sheep's spleen daily.
27.7.30	0.04	Splenectomy.
29.7.30	0.1	Blood Count R = 6,850,000 Hb = 70% W = 27,800 Polymorphonuclears 75% Lymphocytes 15% Large Mononuclears 3% Eosinophils 7%
31.7.30	0.05	
2.8.30	0.4	
4.8.30	0.72	
5.8.30	2.0	Blood Count R = 7,350,000 Hb = 70% W = 28,200 Polymorphonuclears 81% Lymphocytes 13% Large Mononuclears 2% Eosinophils 4%
6.8.30	2.5	
7.8.30	4.4	
8.8.30	4.8	
9.8.30	7.6	
10.8.30	8.5	

No. 23 (continued).

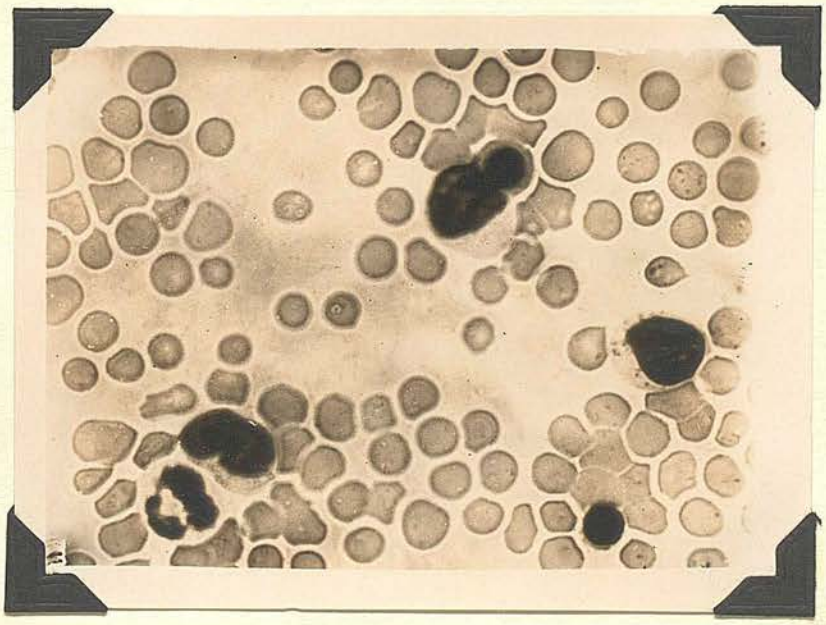
Date	% of Red Cells infected	
11.8.30	19.0	Blood Count R = 7,500,000 Hb = 70% W 30,000 Polymorphonuclears 80% Lymphocytes 15% Large Mononuclears 3% Eosinophils 2%
12.8.30	20.7	Blood Count R = 7,200,000 Hb = 65%
13.8.30	26.0	Blood Count R = 6,250,000 Hb = 60% W = 40,200 Polymorphonuclears 68% Lymphocytes 26% Large Mononuclears 5% Eosinophils 1%
14.8.30	33.0	Blood Count R = 6,00,0000 Hb = 60% Urine, much albumen.
15.8.30	44.0	Blood Count R = 5,850,000 W = 55,400 Polymorphonuclears 57% Lymphocytes 38% Large Mononuclears 2% Eosinophils 3% Blood picture is that of a severe anaemia. Aniscytosis, poikilocytosis and basophilia marked. Megaloblasts and normoblasts present. Some blood was drawn off for a Van den Berg test which was negative.
16.8.30	56.0	Blood Count R = 5,250,000 Hb = 60% Blood picture as before. Urine: brownish, trace of oxyhaemoglobin for the first time.
17.8.30	68.0	Blood Count R = 4,050,000 Hb = 60% Blood picture as before. Urine: clear, no blood, but albuminuria still heavy.

No. 23 (continued).

Date	% of Red Cells infected.	
18.8.30	70.0	Blood Count R = 4,250,000 W = 40,600 Polymorphonuclears 54% Lymphocytes 36% Large Mononuclears 6% Eosinophils 4% Blood picture as before. Urine : trace of blood pigment.
19.8.30	73.0	Blood Count R = 3,600,000 Hb = 40% Blood picture as before Urine : no blood pigment.
20.8.30	75.0	Blood Count R = 2,650,000 Hb = 40% W = 41,400 Blood picture as before. Urine : smoky brown, oxyhaemoglobin present.
21.8.30	57.0	Blood Count R = 2,650,000 Hb = 40% Blood picture as before. Urine : port- wine coloured. Oxyhaemoglobin present.
22.8.30	53.0	Blood Count R = 2,550,000 Hb = 40% Blood picture as before Urine : clear, albuminuria but no haemoglobinuria.
23.8.30	41.0	Blood Count R = 1,450,000 Hb = 35% Blood picture as before Urine as before.
24.8.30	24.0	Blood Count R = 1,850,000 W = 52,200 Polymorphonuclears 42% Lymphocytes 54% Large Mononuclears 3% Eosinophils 1% Blood picture as before. Urine as before.

No. 23 (continued).

Date	% of Red Cells infected.	
25.8.30	22.0	Blood Count R = 2,850,000 Hb = 40% Blood picture and urine as before.
26.8.30	18.0	Blood Count R = 3,100,000 Hb = 50% Blood picture and urine as before.
27.8.30	11.0	Blood Count R = 3,250,000 Hb = 55% Blood picture as before but abnormal features not so marked. Urine, as before.
28.8.30	8.7	Blood Count R = 4,150,000 Hb = 55% W = 42,200 Polymorphonuclears 53% Lymphocytes 38% Large Mononuclears 5% Eosinophils 4% Blood picture and urine as before.
30.8.30	7.6	Blood Count R = 4,100,000 Hb = 55% Blood picture and urine as before.
1.9.30	5.6	Blood Count R = 4,550,000 Hb = 60% Blood picture : anaemia much improved. No megaloblasts or normoblasts. Urine, clear, albuminuria still present.
3.9.30	4.8	Blood Count R = 4,750,000 Hb = 60%
5.9.30	4.5	Blood Count R = 4,800,000 Hb = 60%
7.9.30	4.2	
9.9.30	4.3	Blood Count R = 4,850,000 Hb = 60%



Figures 2 and 3.

Microphotographs of blood films of Cats No. 23 on 23.8.30, showing granules (possibly fragments of babesia) within the cytoplasm of mononuclear leucocytes. The severity of the anaemia is illustrated by the presence of aniscytosis, the megaloblasts and a normoblast. (× 900).

Cat No. 31.

Female, black, 1540 gms.

Date	% of Red Cells infected.	
11.7.29		Inoculated subcutaneously with 0.2 cc citrated blood from No. 24.
4.11.29	0.1	
14.1.30	0.02	
5.4.30	0.03	
26.7.30	0.01	
27.7.30	0.02	Splenectomy. Cat =1710 gms, spleen = 3.7, ratio = .23%.
29.7.30	0.1	Blood Count R = 8,600,000 Hb. = 70%. W = 19,600 Polymorphonuclears 69% Lymphocytes 27% Large Mononuclears 2% Eosinophils 2%
30.7.30	0.2	
2.8.30	0.2	
3.8.30	0.8	Blood Count R = 9,100,000 W = 18,400
4.8.30	0.8	
5.8.30	1.4	Blood Count R = 7,300,000 Hb. = 70%. W = 21,200 Polymorphonuclears 71% Lymphocytes 25% Large Mononuclears 3% Eosinophils 1%
6.8.30	2.3	
7.8.30	3.3	
8.8.30	4.9	
9.8.30	6.8	
10.8.30	9.2	
11.8.30	16.3	Blood Count R = 7,250,000 Hb. = 70%. W = 27,400 Polymorphonuclears 67% Lymphocytes 27% Large Mononuclears 1% Eosinophils 5%
12.8.30	17.0	

Cat No. 31 (continued)

Date	% of Red Cells infected.	
13.8.30	18.0	Blood Count
14.8.30	25.0	R = 7,400,000 Hb. = 70% W = 30,800 Polymorphonuclears 62% Lymphocytes 30% Large Mononuclears 4% Eosinophils 4%
15.8.30	42.0	Blood Count R = 6,150,000 Hb. = 60% W = 27,000
16.8.30	47.0	Blood Count R = 6,200,000 Hb. = 60%
17.8.30	43.0	Blood Count R = 5,650,000 Hb. = 60% W = 25,000
18.8.30	61.0	Blood Count R = 4,950,000 Hb. = 60%
19.8.30	66.0	Blood Count R = 3,950,000 Hb. = 50%
20.8.30	63.0	Blood Count R = 3,350,000 Hb. = 40%
21.8.30	50.0	Blood Count R = 2,350,000 Hb. = 40% Aniscytosis marked. Megaloblasts and Normoblasts seen for the first time.
22.8.30	41.0	Blood Count R = 1,650,000 Hb. = 30% Blood picture as above.
23.8.30	30.0	Blood Count R = 1,250,000 Hb. = 30% Aniscytosis very marked. Numerous normoblasts and megaloblasts.

The cat died at 6.30 p.m. The urine was examined daily during the last fortnight but no blood or blood pigment was detected. Albuminuria was a constant feature.

The serum gave a negative Van den Berg reaction, direct and indirect.

An autopsy was performed within half-an-hour of

death. There was no evidence of jaundice. The liver was large, pale and speckled. The kidneys were pale. The liver and kidneys both gave a strong Prussian Blue reaction. The bone-marrow of the femur was jelly-like and deep red in colour. No other pathological features were noted. Sections of the organs were put up in Bouin's fixative; and examined microscopically. The liver exhibited cloudy swelling and fatty degeneration particularly marked on the periphery of the lobules. In the kidney, cloudy swelling and fatty degeneration were present in the tubular epithelium. The glomeruli appeared to be healthy. In both organs pigment deposits were present within the cells. The section of bone-marrow appeared to be very cellular and the picture was that of an erythroblastic reaction.

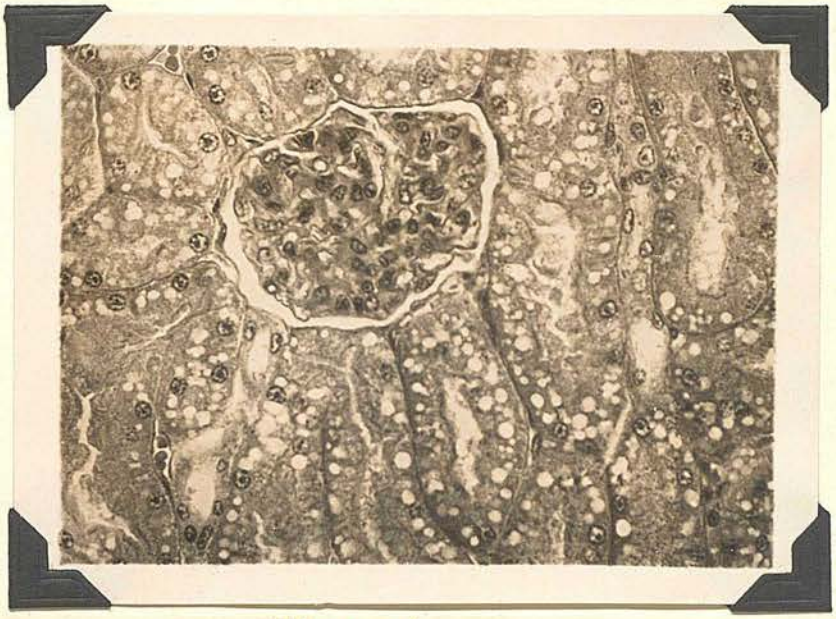


Figure 4.
Microphotograph of section of kidney, Cat No.31. (× 350).

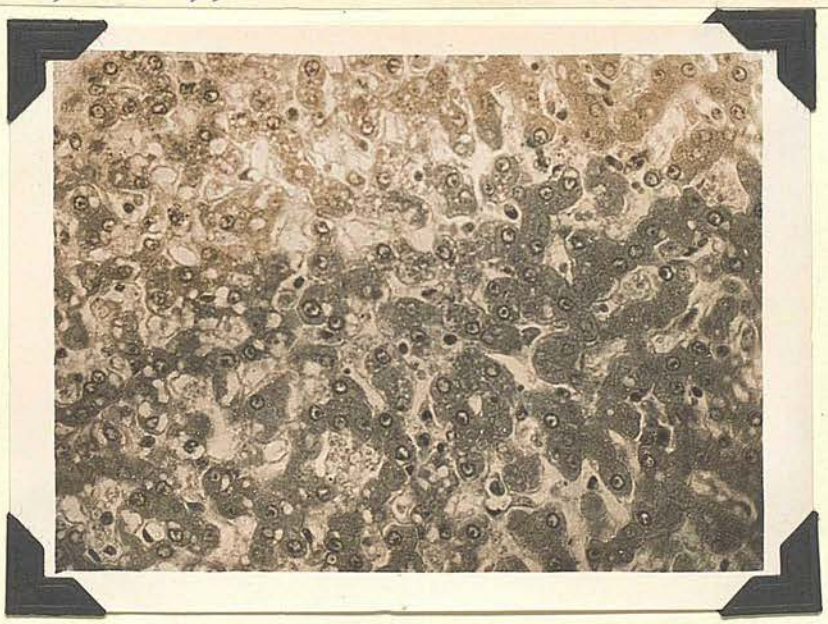
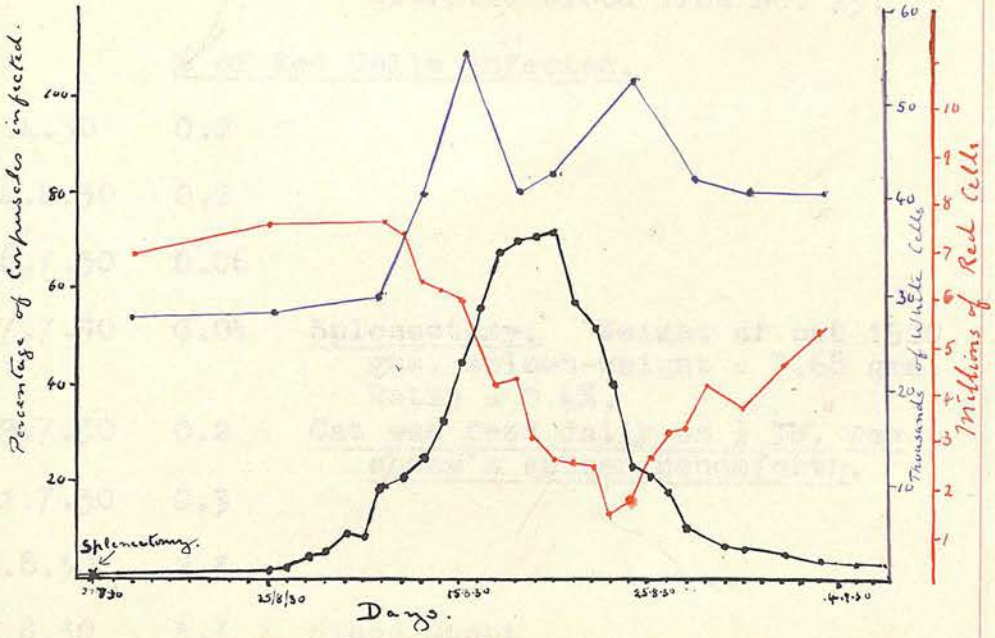


Figure 5.

Microphotograph of section of liver, Cat No. 31 (× 300)
These two photographs illustrate the fatty degeneration and cloudy swelling.

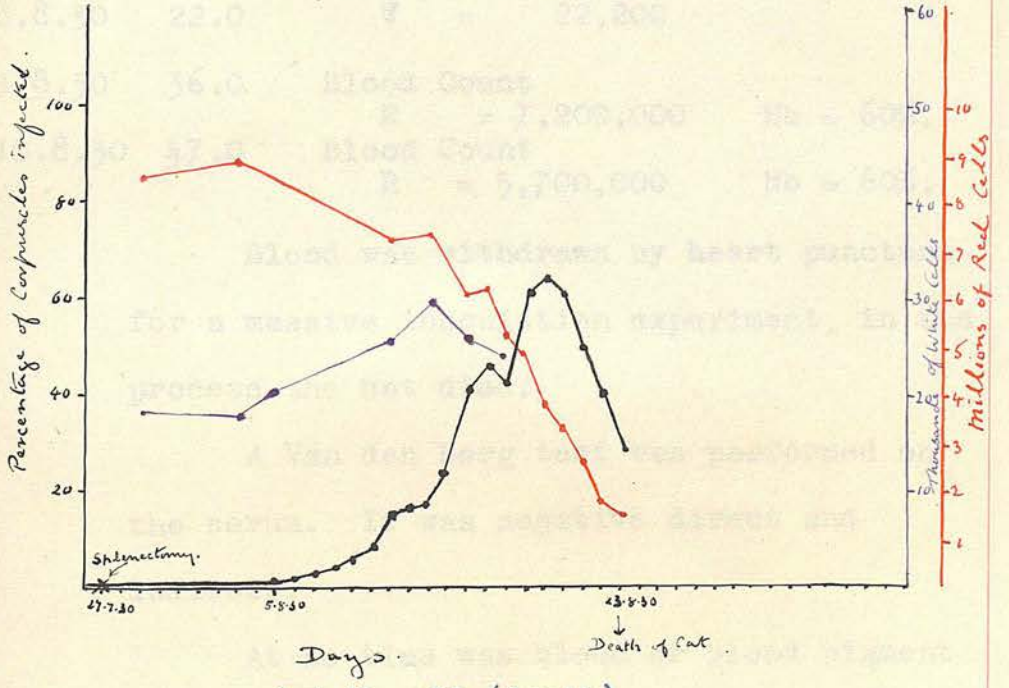


Cat No. 23 (recovery)

= Degree of infection

= Red cell-count

= White cell-count.



Cat No. 31 (death).

Cat No. 39.

Male, black and white, 1750 gms.

Date	% of Red Cells infected.	
15.1.30		Inoculated subcutaneously with 0.5 cc citrated blood from No. 23.
5.4.30	0.2	
14.4.30	0.2	
26.7.30	0.06	
27.7.30	0.04	Splenectomy. Weight of cat 1920 gms. spleen-weight = 7.68 gms. Ratio = 0.4%.
29.7.30	0.2	Cat was feed daily on ¼ lb. raw sheep's spleen henceforth.
31.7.30	0.9	
2.8.30	2.3	
3.8.30	3.3	Blood Count R = 7,750,000 Hb = 70%.
4.8.30	4.1	W = 22,400
5.8.30	7.5	
6.8.30	9.5	
7.8.30	14.2	Blood Count R = 7,900,000 Hb = 70%.
8.8.30	22.0	W = 22,200
9.8.30	36.0	Blood Count R = 7,200,000 Hb = 60%.
10.8.30	47.0	Blood Count R = 5,700,000 Hb = 60%.

Blood was withdrawn by heart puncture for a massive inoculation experiment, in the process the cat died.

A Van den Berg test was performed on the serum. It was negative direct and indirect.

At no time was blood or blood pigment observed in the urine.

Post-mortem examination revealed no morbid changes.

Cats Nos. 12, 21, 24, 47 & 50.

Infected after Splenectomy.

Cat No. 12.

Male, White and tabby, 2100 gms.

Date		
1.12.28	Blood Count	Temp. 101.9 °F
	R = 8,250,000	
	W = 16,800	
	Polymorphonuclears	71%
	Lymphocytes	22%
	Large Mononuclears	4%
	Eosinphils	3%
	H-J. bodies in 0.12% of corps.	
	Splenectomy performed.	
	% of Red Cells infected.	
6.12.28	0	
10.12.28	0	
15.12.28	0	H-J. bodies in 0.14% of corps.
19.12.28	0	
26.12.28	0	Blood Count Temp. 101.8 °F.
	R = 8,000,000	
	W = 17,000	
	Polymorphonuclears	70%
	Lymphocytes	23%
	Large Mononuclears	5%
	Eosinophils	2%
	H-J. bodies in 0.1% of corps.	
1.1.29	0	Inoculated subcutaneously with 0.5cc citrated blood from No. 13.
5.1.29	0	
8.1.29	0	
12.1.29	0	H-J. bodies in 0.1% of corps.
15.1.29	0	
17.1.29	0.1	
19.1.29	0.8	
22.1.29	15.2	
24.1.29	75.0	Blood Count Temp. 100.8 °F.
	R = 4,950,000	
	W = 19,000	
	Polymorphonuclears	75%
	Lymphocytes	18%
	Large Mononuclears	4%
	Eosinophils	3%
	Urine drawn off. reddish in colour, contained oxyhaemoglobin and albumen. No blood corpuscles and no bile pigments.	

No. 12 continued.

Date	% of Red Cells infected.	
25.1.29	95.0	Blood Count Temp. 101.2 °F. R = 1,900,000 W = 20,200 Urine, as before.

The cat died during the night of the 25th., Post-mortem findings : Free fluid in the peritoneum, Intestines were bile stained.

Liver was studded with small white areas and appeared very fatty. On microscopical examination, areas of focal necrosis were found. Cloudy swelling and fatty degeneration were marked features. Deposits of pigment were numerous.

The Kidneys appeared engorged. Microscopically the vessels appeared congested with blood cells, the spaces between the glomeruli and the capsules were filled with plasma. The cytoplasm exhibited obvious cloudy swelling. The bladder contained reddish urine, which was found to contain epithelial cells, oxy-haemoglobin and albumen.

The marrow of the long bones appeared to be very red in colour, and microscopically exhibited the erythroblastic reaction.

Smears and sections of all the organs were searched for evidence of schizogony forms of the parasite, but with negative results.

Cat No. 21.

Male, black and white, 1750 gms.

Date	% of Red Cells infected.	
7.5.29	0	Splenectomy performed. Blood Count R = 7,350,000 Hb = 70% W = 17,200 Polymorphonuclears 68% Lymphocytes 26% Large Mononuclears 4% Eosinophils 2% H-J. bodies in 0.1% of corpuscles.
23.5.29	0	Inoculated subcutaneously with 1 cc. citrated blood from N. 6.
9.6.29	0	
17.6.29	0	
25.6.29	0.1	
3.7.29	25.0	Blood Count R = 7,000,000 Hb = 70% W = 20,000
4.7.29	50.0	Blood Count Temp. = 102.8 °F. R = 7,200,000 Hb = 70% W = 18,800 Normoblasts present.
5.7.29	60.0	Blood Count Temp. 101.9 °F. R = 5,000,000 Hb = 50% W = 18,000 Normoblasts present. Urine port-wine coloured, oxyhaemoglobin albumen. No bile pigment and no corpuscles.
6.7.29	65.0	Blood Count Temp. 100.8 °F. R = 2,900,000 Hb = 40% W = 21,000 Normoblasts present. Urine as above plus a trace of bilirubin.
7.7.29	80.0	Blood Count Temp. 98.5 °F. R = 1,900,000 Hb= 30% W = 16,000 Polymorphonuclears 66% Lymphocytes 27% Large Mononuclears 6% Eosinophils 1% Normoblasts and megaloblasts present, aniscytosis marked.

No. 21. continued.

Date	% of Red Cells infected.	
8.7.29	68.0	Blood Count Temp. 97.8 °F. R = 1,800,000 W = 16,400 Normoblasts & megaloblasts present. Urine brown coloured, contained oxyhaemoglobin, albumen, a trace of bile pigment and a few epithelial cells. No red blood corpuscles.

The cat died at 2.30 p.m..
At autopsy the only marked changes, naked-eye, were the pale greasy appearance of the liver, which gave a strong Prussian blue reaction, and the red gelatinous appearance of the femur marrow.

Microscopically, cloudy swelling and fatty degeneration were apparent in the liver cells and the tubular epithelial cells of the kidneys. The femur marrow presented an erythroblastic appearance.

Cat No. 24.

Female, Black and white, 1450 gms.

Date	% of Red Cells infected.	
7.5.29	0	Blood Count Temp. 102.2 °F. R = 7,250,000 Hb =70% W = 14,800 Polymorphonuclears 74% Lymphocytes 19% Large Mononuclears 2% Eosinophils 5% Splenectomy performed.
23.5.29	0	Inoculated subcutaneously with 0.2 cc of citrated blood from No. 6.
9.6.29	0	Blood Count Temp. 101.8 °F. R = 6,950,000 Hb=70% W = 17,000 Polymorphonuclears 72% Lymphocytes 21% Large Mononuclears 3% Eosinophils 4%
17.6.29	0	
25.6.29	0.2	
3.7.29	1.5	Blood Count Temp. 102.0 °F. R = 6,700,000 Hb=70% W = 18,200
4.7.29	2.2	Blood Count R = 6,850,000 Hb=70%
5.7.29	10.0	Blood Count R = 7,200,000 Hb=70% W = 19,000 Urine - no blood pigment, a trace of albumen present.
6.7.29	14.0	Blood Count Temp. 101.8 °F. R = 7,150,000 Hb=70% W = 17,400
7.7.29	20.0	Blood Count R = 5,500,000 Hb=60% W = 18,600 Temp. 102.2 °F. Urine - trace of oxyhaemoglobin, albumen present.
8.7.29	22.0	Blood Count Temp. 103.1 °F. R = 5,100,000 Hb=60% W = 18,200 Urine - clear, oxyhaemoglobin spectroscopically no albumen.
9.7.29	25.0	Blood Count Temp. 101.2 °F. R = 3,750,000 Hb=50% W = 31,000 No normoblasts present. Urine slight brown in colour, oxyhaemoglobin and albumen present.

No. 24 continued.

Date	% of Red Cells infected.	
10.7.29	30.0	Blood Count Temp. 101.1 °F. R = 3,200,000 Hb= 40% W = 32,000 Urine - brown coloured, no blood corpuscles. Oxyhaemoglobin and albumen present.
11.7.29	24.0	Blood Count Temp. 102 °F. R = 2,250,000 Hb= 30% W = 44,600 Polymorphonuclears 67% Lymphocytes 27% Large Mononuclears 3% Eosinophils 3% Normoblasts present. Urine, clear, no blood pigment, no bile pigment, faint trace of albumen.
12.7.29	22.0	Blood Count Temp. 102.2 °F. R = 2,100,000 Hb= 30% W = 42,800 Normoblasts present Urine - no blood or bile pigment, faint trace of albumen.
No further examination possible for three months		
4.11.29	2.3	Blood Count Temp. 102.2 °F. R = 5,500,000 Hb= 60% W = 32,000 Polymorphonuclears 69% Lymphocytes 27% Large Mononuclears 2% Eosinophils 2% H-J bodies present in 0.16% of corpuscles. Urine - no blood or bile pigment, faint trace of albumen.
8.12.29	1.8	
29.12.29	2.2	

Cat died two months later during writer's absence.

Cat No.47

Male, black, 1950 gms.

Date	% of Red Cells infected.	
27.7.30	0	Splenectomy performed.
6.8.30	0	Inoculated subcutaneously with 0.3cc citrated blood from No. 39.
17.8.30	0	Blood Count
		R = 6,750,000 Hb = 70%
21.8.30	0	W = 17,400
		Polymorphonuclears 71%
23.8.30	0	Lymphocytes 24%
		Large Mononuclears 2%
25.8.30	0	Eosinophils 3%
27.8.30	0.3	
28.8.30	1.4	
29.8.30	2.2	
30.8.30	3.0	Blood Count
		R = 5,950,000 Hb = 70%
31.8.30	8.0	W = 24,200
1.9.30	9.0	Blood Count
		R = 4,050,000 Hb = 60%
		W = 28,600
		Polymorphonuclears 72%
		Lymphocytes 25%
		Large Mononuclears 2%
		Eosinophils 1%
2.9.30	10.0	Blood Count
		R = 3,400,000 Hb = 40%
3.9.30	16.0	Blood Count
		R = 3,200,000 Hb = 40%
		W = 35,200
		Polymorphonuclears 64%
		Lymphocytes 32%
		Large Mononuclears 3%
		Eosinophils 1%
4.9.30	18.0	Blood Count
		R = 2,950,000 Hb = 40%
		Blood picture : nucleated reds and megaloblasts present, aniscytosis marked.
		Urine, albumen present, but no oxyhaemoglobin.
5.9.30	24.0	Blood Count
		R = 1,700,000 Hb = 40%
		W = 29,200
		Blood picture and Urine as before.

No. 47 (continued).

Date	% of Red Cells infected.	
6.9.30	31.0	Blood Count R = 1,750,000 Hb = 40% Blood picture and urine as before.
7.9.30	43.0	Blood Count R = 1,350,000 Hb = 30% W = 22,200 Polymorphonuclears 77% Lymphocytes 18% Large Mononuclears 4% Eosinophils 1% Blood picture : megaloblastic anaemia of severe type. Urine - no blood pigment.

Cat died later in some day. An autopsy was performed immediately. The chief morbid changes were the pale, fatty liver and kidney both of which gave a Prussian-blue reaction, and the red jelly-like marrow in the femur.

Cat No. 50.

Male, black, 1710 gms.

Date	% of Red Cells infected.	
28.7.30	0	Splenectomy
30.7.30	0	Inoculated subcutaneously with 0.75 cc citrated blood from No. 32.
31.7.30	0	Henceforth ¼ lb. raw sheep's spleen was fed daily.
3.8.30	0	Blood Count
5.8.30	0	R = 6,600,000 Hb = 70% W = 21,000 Polymorphonuclears 82% Lymphocytes 14% Large Mononuclears 2% Eosinophils 2%
7.8.30	0	
9.8.30	0	
11.8.30	0	
13.8.30	0	Blood Count
15.8.30	0	R = 6,950,000 Hb = 70% W = 34,000 Polymorphonuclears 83% Lymphocytes 15% Large Mononuclears 0 Eosinophils 2%
17.8.30	0	
19.8.30	0	
21.8.30	0	
23.8.30	0.02	
25.8.30	0.12	
27.8.30	0.8	
28.8.30	5.4	
30.8.30	12.0	Blood Count
		R = 6,800,000 Hb = 60% W = 18,400
1.9.30	15.0	Blood Count
		R = 1,700,000 W = 19,000 Polymorphonuclears 54% Lymphocytes 36% Large Mononuclears 9% Eosinophils 1%

No. 50 (continued).

Blood picture is that of a severe anaemia, aniscytosis and basophilia marked. Megaloblasts and normoblasts present. Urine : albuminuria, haemoglobinuria.

The cat died later in the day. At post-mortem examination few morbid changes were noted. The marrow of the long bones afforded evidence of a commencing erythroblastic reaction. Liver and kidneys failed to give the Prussian Blue reaction.

The Effect of Reticulo-endothelial Blocade.

Two normal cats chronically infected with Babesia felis were subjected to daily intravenous injections of India ink with the object of "blocading" the reticulo-endothelial system. The ink used was Windsor and Newton's carbon ink suspended in isotonic saline. It was injected into the leg veins of the animals.

The appended protocols of these cats-Nos. 40 and 45 - show that this "blocade" was quite without effect on the degree of intensity of infection. Possibly the dosage was inadequate, although that administered to No. 45 will be seen to be considerable.

Cat No. 40.

Female, white, 1550 gms.

Date				
15.1.30	Inoculated subcutaneously with 0.5 cc citrated blood from No. 23.			
	No examination possible for three months.			
	% of Red Cells infected.			
14.4.30	0.3			
26.7.30	0.2			
28.7.30	0.3	1 cc 15%	Suspension India Ink injected	intravenously.
29.7.30	0.3	1.5 cc	ditto.	ditto.
30.7.30	0.3	"	"	"
31.7.30	0.3	"	"	"
1.8.30	0.2	2 cc	"	"
2.8.30	0.2	"	"	"
3.8.30	0.3	"	"	"
4.8.30	0.3	"	"	"
5.8.30	0.3	"	"	"
6.8.30	0.6	"	"	"
9.8.30	0.03	"	"	"
10.8.30	0.1	"	"	"
11.8.30	0.2	"	"	"
12.8.30	0.3	"	"	"
16.8.30	0.4	Blocade stopped.		
20.8.30	0.4			
27.8.30	0.4			
31.8.30	0.4			
7.9.30	0.3			

Cat No. 45.

Male, black and white 1600 gms.

Date	% of Infected Red Cells.	
14.4.30		Inoculated subcutaneously with 0.2 cc citrated blood from No. 31.
26.7.30	0.1	
28.7.30	0.2	3 cc 20% suspension India Ink injected intravenously.
29.7.30	0.2	" " "
30.7.30	0.1	" " "
31.7.30	0.1	" " "
1.8.30	0.2	" " "
2.8.30	0.2	" " "
3.8.30	0.4	" " "
4.8.30	0.1	" " "
5.8.30	0.1	" " "
6.8.30	0.2	" " "
9.8.30	0.01	Blocade stopped.
10.8.30	0.01	
12.8.30	0.01	
16.8.30	0.03	
20.8.30	0.04	
27.8.30	0.06	
31.8.30	0.06	
7.8.30	0.05	

The Possibility of Oral Transmission of Babesia felis.

In view of the successful experimental transmission of Kala Azar by feeding monkeys and hamsters on infected material recorded respectively by Archibald (1914), and Shortt and colleagues (1929), it was deemed worth while investigating the possibility of a similar mode of transmission of feline piroplasmosis.

Spleenless cats were considered particularly suitable for such experiments as in their case there would little chance of an infection being missed once it became established. Cats Nos. 58, 59 and 60 were splenectomized and subsequently given four consecutive daily doses of citrated blood from a heavily infected cat. The blood was dropped into the animals' mouths from a pipette and was seen to be actually swallowed. In no case did infection ensue within the succeeding six weeks.

Date	Cats. 58	59	60
28.7.30	No parasites present. Splenectomised.		
1.8.30	Fed on 1 cc. citrated blood from No. 39.		
2.8.30	"	"	"
3.8.30	"	"	"
4.8.30	"	"	"
14.8.30	No parasites		present
19.8.30	"	"	"
23.8.30	"	"	"
31.8.30	"	"	"
5.9.30	"	"	"
12.9.30	"	"	"

III

Experimental Feline Trypanosomiasis

A preliminary observation made last year on the fate of a spleenless cat infected with Trypanosoma brucei compared with that of a normal control suggested that the latter had definitely more resistance. It was accordingly decided to investigate the question further.

The susceptibility of cats to T.brucei is well known. Kanthack, Durham and Blandford (quoted by Laveran and Mesnil 1907) give the incubation period as five days, and state that death occurs in twenty-two to twenty-six days, they further remark that the parasites are present in the blood but show marked daily fluctuations. Attention has already been drawn to Bradford and Plimmer's claims that splenectomy of the cat hastens the fatal issue in this infection. They state that a spleenless cat died in twelve days against average of twenty in the case of control animals.

A strain of T.rhodesiense being available it was decided to compare its pathogenicity for the cat with that of T.brucei. Wenyon (1926) states that in its effect on animals T.rhodesiense differs in no way from T.brucei. This strain known as the Tembura strain, was originally isolated in 1926 from a case of sleeping sickness in the Southern Sudan. The original discovery of T.rhodesiense in

the Sudan was recorded by Archibald (1922). The strain of T.brucei was isolated from a cow in the Nuba Mountains Province of the Sudan in 1927. Both strains have since been maintained in white rats and latterly in gerbils.

Four cats were inoculated with each strain. Two cats in each series had their spleens extirpated six days previously. Each cat was inoculated subcutaneously with 0.1 cc of a citrated suspension of gerbil's blood containing one hundred thousand trypanosomes per cubic millimetre. During the ensuing month daily records were made of the intensity of trypanosome infection in the blood of each cat. The trypanosome counts were made according to the method of Kolmer (1915). The blood was diluted with the following mixture :-

Formalin	2 cc.
Glacial Acetic ac.	2 cc.
Distilled water	96 cc.
Carbol Fuchsin	2 cc.

The dilution was made in an ordinary blood pipette, a "red" or "white" one was chosen according to the intensity of infection. The counts were made in a Thoma-Zeiss counting chamber. In the following tables the figures indicate the number of trypanosomes per cu. mm. of blood. When the numbers were few a single plus sign is used.

It will be seen that in all the cats

the infection pursues a fluctuating sub-acute course indicating that there is a definite resistance. There is an indication that the multiplication of T.brucei in the early stages is less restricted in the spleen-less cats than in the controls. With the small numbers of animals involved however, the data submitted would hardly be of statistical significance. The danger of drawing sweeping conclusions from experiments on single animals is well exemplified in the T.brucei series, where by fortunate chance a normal cat and a spleenless cat both die on the eleventh day after infection, while another control and spleenless cat both survive twenty-eight days.

It does appear permissable however to conclude from these experiments that preliminary splenectomy does not materially affect the resistance of cats to T.brucei and T.rhodesiense infections, and that the operation has no marked influence on the duration of the disease.

Date	Cats 53	54	67	68
29.7.30	Splenectomy			
5.8.30	Inoculated Subcutaneously T.rhodesiense (10,000 trypanosomes)			
8.8.30	0	(+)	(+)	(+)
10.8.30	(+)	(+)	26,000	36,000
11.8.30	28,000	42,000	34,000	48,000
12.8.30	45,000	62,000	400	40,000
13.8.30	32,000	20,000	3,200	52,000
14.8.30	6,600	15,200	55,000	50,000
15.8.30	(+)	1,800	66,000	72,000
16.8.30	4,600	7,000	15,000	32,000
17.8.30	10,200	14,200	Dead	1,800
18.8.30	22,000	20,000		20,000
19.8.30	80,000	48,000		16,600
20.8.30	56,000	72,000		(+)
21.8.30	Dead	150,000		0
22.8.30		204,000		(+)
23.8.30		78,000		(+)
24.8.30		Dead		Dead

Date	Cats No. 52	56	69	70
29.7.30	Splenectomy		-	-
5.8.30	Inoculated subcutaneously T.brucei (10,000 trypenosomes each).			
8.8.30	(+)	(+)	0	(+)
10.8.30	89,000	51,000	1,500	(+)
11.8.30	85,000	78,000	(+)	(+)
12.8.30	42,000	17,000	(+)	0
13.8.30	(+)	6,000	14,000	(+)
14.8.30	1,800	33,000	(+)	12,600
15.8.30	46,000	67,000	(+)	27,000
16.8.30	140,000	Dead	600	Dead
17.8.30	18,000		(+)	
18.8.30	1,200		18,000	
19.8.30	1,800		64,000	
20.8.30	4,600		74,000	
21.8.30	0		18,600	
22.8.30	(+)		28,400	
23.8.30	(+)		34,000	
24.8.30	(+)		68,000	
25.8.30	400		1,400	
26.8.30	1,600		600	
27.8.30	1,200		0	
28.8.30	19,000		0	
29.8.30	43,000		0	
30.8.30	138,000		1,200	

(continued)

Date	Cats No. 52	(56)	69	(70)
31.8.30	216,000		16,200	
1.9.30	240,000		33,000	
2.9.30	350,000		1,700	
3.9.30	Dead		1,200	
4.9.30			(+)	
5.9.30			4,800	
6.9.30			29,000	
7.9.30			83,000	
8.9.30			Dead	

IV

Haemogregarina Balfouri in the Jerboa

The only reference in the available literature to the effect of splenectomy on haemogregarine infections is the paper by Nauck (1927) who reported that splenectomy of squirrels activated a haemogregarine infection.

In the Sudan the majority of the desert jerboas (Jaculus jaculus. Thomas 1903) harbour the haemogregarine originally described by Balfour (1905) and named by Laveran, Haemogregarina balfouri. It was decided to observe the effect of splenectomy of the jerboa on the intensity of haemogregarine infection.

A dozen infected jerboas were accordingly operated on. The intensity of infection of each was recorded before and after operation. Using Leishman stained blood-films of uniform thickness the number of haemogregarines observed in twenty fields of the microscope was counted on each occasion. A 1/12th inch oil immersion objective with a X 6 ocular was employed. This method while not strictly accurate, was considered adequate for the observation of any significant alteration in the intensity of infection. It was of course essential to work only with satisfactory films. The splenectomies were performed under ether anaesthesia.

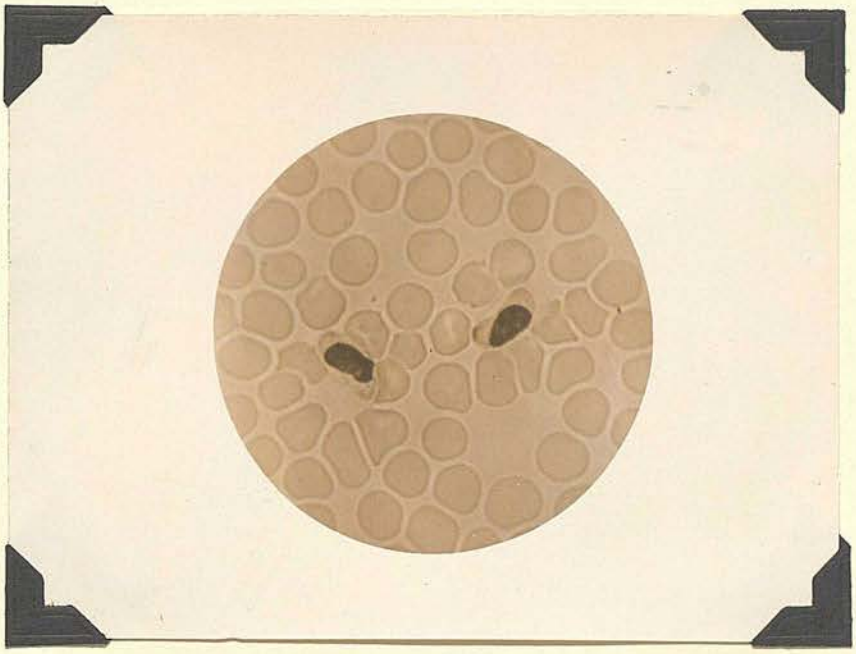


Figure 6.

Microphotograph of blood film of Jerboa, showing Haemogregarina balfouri.(× 1200).

At the operation the animals and the spleens were weighed in order to determine the ratio between the spleen-weight and body-weight in each case. This was found to be low . It varied between .02% and .03%.

The results of the experiment are appended in the accompanying tables. It will be seen that splenectomy of the jerboa had no significant effect on the numbers of haemogregarines infecting it. The very small size of the spleen in the jerboa relative to the body weight is probably a significant factor, in that by splenectomy only a small proportion of the total reticulo-endothelial system of the animal's body is removed.

DATE	JERBOAS					
	1	2	3	4	5	6
12.1.29	18	21	3	7	9	10
25.2.29	23	22	5	6	13	8
	SPLENECTOMY					
26.2.29	.02/67	.02/52	.01/33	.01/27	.02/69	.01/31
28.2.29	21	23	7	D	21	13
2.3.29	24	21	4		20	14
6.3.29	27	20	9		23	12
12.3.29	23	18	8		27	17
26.3.29	18	22	12		26	15
14.4.29	14	20	10		23	14

The figures indicate the number of haemogregarines observed in twenty microscopic fields on each occasion.

The fractions opposite the date of splenectomy indicate the weight of the spleen over the weight of the jerboa in grammes.

DATE	JERBOAS					
	7	8	9	10	11	12
12.1.29	53	442	12	6	20	18
25.2.29	48	463	6	11	17	15
	SPLENECTOMY					
26.2.29	.01 / 24	.02 / 58	.02 / 65	.01 / 34	.01 / 29	.01 / 30
28.2.29	D	457	7	7	D	13
2.3.29		461	6	10		11
6.3.29		452	5	10		17
12.3.29		456	9	13		25
26.3.29		572	13	14	D	D
14.4.29		551	14	19		

V

Grahamella Bodies in the Gerbil.

Graham-Smith (1905) studying stained blood-films of the English mole observed that certain of the red cells were occupied by bacilliform bodies. Similar structures have since been found in many small mammals. In the Sudan they have been recorded in the jerboa (Balfour 1906) and in gerbil (Balfour 1911). Brumpt (1911) studied these bodies, concluded that they were parasites and created for them the genus Grahamella. Laveran and Maraullaz (1914) however, submitted that the structures were not living organisms but that they represented a change in the red cell analogous to basophilic degeneration. Brumpt (1928) again studied the question and re-affirmed that the bodies are living parasites, and further, he has compared Grahamella muris of the brown rat with Bartonella muris of the desplenated white rat and considers that the two belong to the same genus. He accordingly advocates the suppression of the name Bartonella in favour of Grahamella.

The recent work on the effect of splenectomy on Bartonella infections to which reference has already been made, suggests that if Grahamella bodies are living parasites akin to Bartonella, which they undoubtedly morphologically resemble, then splenectomy of the infected animal

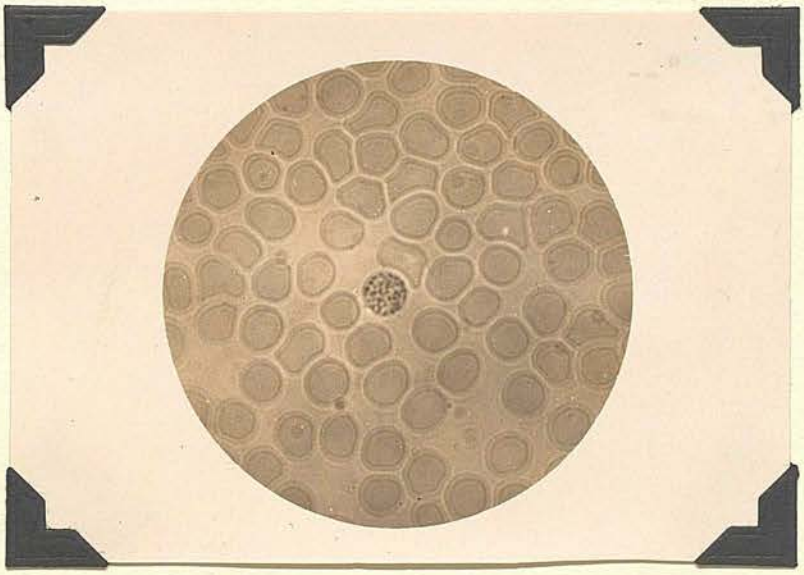


Figure 7.

Microphotograph of blood film of Gerbil, showing a corpuscle with Grahamella bodies. (x 1200).

host might be expected to influence the relations existing between it and its Grahamella. At all events if splenectomy did lead to a significant increase in the number of affected red cells it would lend strong support to the view that Grahamella bodies are living parasites.

A number of Egyptian gerbils or desert rats (Gerbillus aegyptius, Desmarest 1804) were available. In approximately sixty per cent of these, Grahamella bodies were readily found in Leishman stained films. Shousha and Aly (1928) record that the Grahamella bodies of the Egyptian gerbil damage the erythrocytes, but the writer saw no evidence of this. The affected corpuscles were never numerous, they varied from one to twenty three in ten thousand cells. Generally the affected erythrocytes stained slightly more bluish than the neighbouring cells. The plan adopted was to collect a number of gerbils, to observe the degree of "infection" for a short period, to make red blood-counts, and then remove the spleens and observe the effects. Eighteen animals were selected for the experiment. In eleven of these Grahamella bodies were present at the outset. In four no bodies were found at first, but were subsequently; the remaining three never developed the bodies.

The Grahamella counts were carried out by observing in each case fifty fields using a 1/12th inch objective and a X 10 ocular fitted with a square diaphragm. Using only well spread, compact films this method was found reasonably accurate in allowing one to fairly rapidly survey ten thousand corpuscles on each occasion. The blood for making the films was obtained by pricking the dorsal tail vein of the animal.

The splenectomies were performed under ether anaesthesia. The operation presented no difficulty but in five cases the gerbils died within twenty-four hours. Before each operation the animal was weighed and afterwards the spleen was also weighed. The ratio of the spleen-weight to body-weight varied between 0.09% and 0.15%. This is nuch the same as in the cats.

The results of the experiments appear in the following tables. In each case the single or double number represents the number of red-cells containing Grahamella bodies seen in approximately ten thousand corpuscles examined, and the large number represents the red blood-count. The fractions opposite the date of splenectomy represent the ratio of spleen-weight to body-weight.

Examination of the results reveal that splenectomy is followed by no significant

alteration in the number of red corpuscles exhibiting Grahamella bodies, and by no significant anaemia. It would perhaps be overbold to state that these results definitely support the view that the bodies are of an inanimate nature, but the results certainly do not lend support to the parasitic theory.

DATE	GERBILS			
	1	2	3	4
9.1.29	1 9,750,000	3 8,250,000	0 9,350,000	5 10,350,000
20.1.29	0	2	0	7
21.2.29	1	4	0	3
3.3.29	1 10,600,000	1 7,950,000	0 9,750,000	5 10,750,000
5.3.29	SPLENECTOMY 0.03 Gm. 35.7	0.01 Gm. 17.8	0.06 Gm. 46.3	0.07 Gm. 46.5
7.3.29	D	3 6,800,000	0 8,650,000	11 9,250,000
10.3.29		4	0	7
15.3.29		4	0	8
23.3.29		6 8,050,000	0 10,150,000	5 9,450,000
15.4.29		3	0	9
27.4.29		3	D	4
29.5.29		5 9,150,000		7 9,700,000

DATE	GERBILS			
	5	6	7	8
9.1.29	0 11,200,000	11 9,800,000	- -	-
20.1.29	2	8	23 10,730,000	0 8,700,000
21.2.29	1	9	22	2
3.3.29	1 11,300,000	10 10,250,000	17 9,850,000	1 9,350,000
	SPLENECTOMY			
5.3.29	0.04 Gm. 39.2	0.01 Gm. 12.8	0.03 Gm 27.5	0.03 Gm. 25.3
7.3.29	0 12,250,000	D	19 8,735,000	D
10.3.29	5		15	
15.3.29	4		17	
23.3.29	7 11,700,000		19 9,700,000	
15.4.29	2		19	
27.4.29	0		23	
29.5.29	3 10,300,000		25 9,200,000	

DATE	GERBILS			
	9	10	11	12
20.1.29	7 10,300,000	2 7,960,000	13 9,750,000	0 8,800,000
21.2.29	3	0	11	0
3.3.29	4 9,750,000	1 8,300,000	0 10,250,000	0 9,250,000
5.3.29	SPLENECTOMY			
	0.02 Gm. 19.5	0.06 Gm. 42.4	0.05 Gm. 37.8	0.03 Gm. 28.2
7.3.29	D	3 10,450,000	17 7,500,000	0 8,750,000
10.3.29		1	13	0
15.3.29		0	13	0
23.3.29		2 8,650,000	14 9,300,000	0 8,650,000
15.4.29		D	12	0
27.4.29			11	0
29.5.29			5 11,300,000	0 9,150,000

DATE	GERBILS			
	13	14	15	16
6.2.29	4 10,200,000	0 8,850,000	0 11,300,000	17 9,750,000
21.2.29	2	0	2	12
3.3.29	1 9,850,000	0 9,250,000	1 12,100,000	19 10,300,000
5.3.29	SPLENECTOMY 0.04 Gm. 37.6	0.03 Gm. 22.6	0.02 Gm. 14.5	0.06 Gm. 42.2
7.3.29	0 7,360,000	0 5,750,000	D	24 10,250,000
10.3.29	0	0		17
15.3.29	3	0		19
23.3.29	1 9,300,000	D		12 11,200,000
15.4.29	3			7
27.4.29	4			15
29.5.29	1 8,850,000			2 10,800,000

DATE	GERBILS	
	17	18
6.2.29	3	0
	9,650,000	8,700,000
21.2.29	1	2
3.3.29	2	1
	10,200,000	9,350,000
	SPLENECTOMY	
5.3.29	0.03 Gm.	0.04 Gm.
	27.3	39.2
7.3.29	0	3
	6,800,000	8,350,000
10,3.29	2	3
15.3.29	5	2
23.3.29	3	1
	11,300,000	8,700,000
15.4.29	9	1
27.4.29	2	0
29.5.29	4	3
	9,950,000	9,200,000

VI

Summary

1. The rôle of the spleen in immunity in general has been briefly discussed. The effect of experimental splenectomy on infections, particularly those due to protozoa has been considered and the relevant literature reviewed.

2. Experimental feline piroplasmosis, an infection of the domestic cat by Babesia felis Davis, had been studied in eleven cats. It has been shown to follow a uniform benign course, in which at most one per cent of the red blood-cells become parasitized, and to be without any apparent ill-effects on the host.

3. Extirpation of the spleen either prior to infection or subsequently, has been shown to modify the course of feline piroplasmosis most profoundly. Details of observations on the course of infection in eleven spleenless cats have been submitted, and the following conclusions drawn. A rapid multiplication of the parasites resulting in more than fifty per cent of the red cells becoming parasitized, accompanied by a severe megaloblastic anaemia occurred in all cases. Haemoglobinuria was an inconstant feature. Death ensued in more than half the cases studied. Recovery appeared to be associated with a relatively high leucocyte count. The morbid anatomy of the fatal cases has

been described : the chief features were cloudy swelling and fatty degeneration in the liver and kidney and an erythroblastic reaction of the marrow in the long bones.

4. It has been shown that a diet of raw sheep's spleen failed to modify the severity of piroplasmosis in spleenless cats.

5. Attempted "blocade" by means of intra-venous infections of India ink exercised no apparent influence on the course of the infection in normal cats.

6. An unsuccessful attempt to infect spleenless cats with Babesia felis by feeding with infected blood has been described.

7. Trypanosoma brucei and Trypanosoma rhodesiense infections have been studied in normal and in spleenless cats : the infections apparently followed similar courses in both series.

8. Jerboas infected with Haemogregarina balfouri were splenectomized, but no significant modification of the degree of infection was found to occur.

9. Splenectomy of gerbils in which a small percentage of the red blood-cells contained Grahamella bodies did not materially affect the relative number of affected cells, nor did it result in any appreciable variation in the red cell-counts.

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